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EVALUATION OF POTENTIAL ANTIDIABETIC AND ANTIBACTERIAL ACTIVITIES OF BOLIVIAN NUTRACEUTICAL PLANTS

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Evaluation of potential antidiabetic and antibacterial activities of Bolivian nutraceutical plants

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To my beloved Ludwing

"Love the life you live, live the life you love"

- Bob Marley

ABSTRACT

Type 2 diabetes mellitus is a health problem worldwide that requires the search for novel therapeutic strategies. Natural products are a source of potential therapies due to their acceptation and traditional use or consumption. This study aims to evaluate selected Bolivian traditionally consumed plants as potential antidiabetic products using animal models. Besides, focused on the urinary tract infection, as one common complication in diabetes, the antibacterial effect was evaluated in the uroepithelial *in vitro* infection.

Initially, the screening of glycemia-reducing effect, performed in healthy Swiss albino mice, showed that the hydroethanolic extracts of *Amaranthus caudatus*, *Chenopodium quinoa*, *Lupinus mutabilis*, and *Smallanthus sonchifolius* reduced the postprandial glycemia and the glucose tolerance during the oral glucose tolerance test. Moreover, the *in vitro* insulin secretion in mice pancreatic islets was stimulated only by *Amaranthus caudatus*, *Chenopodium quinoa* and *Lupinus mutabilis*. Based on the results of the screening study, we continued evaluating the antidiabetic effect of *Amaranthus caudatus* and *Lupinus mutabilis* using the Goto-kakizaki (GK) type 2 diabetic rats and the respective healthy control the Wistar (W) rats.

The oral administration of *A. caudatus* and *L. mutabilis*, separately, improved the glucose tolerance and augmented serum insulin levels in both GK and W rats, in a dose-dependent way. The daily oral administration of each extract (1000 mg/kg b.w.) during 21 days improved the glucose tolerance, increased the serum insulin and reduced the glycated hemoglobin. Additionally, the insulin secretion was stimulated in islets isolated from treated animals. Furthermore, both extracts stimulated the *in vitro* insulin secretion in a glucose-independent manner. In perifused islets, the insulin secretion was augmented in low glucose (3 mM), increased gradually in high glucose (16.7 mM), and was restored to basal levels when each extract was removed. The mechanism behind the stimulation of insulin secretion of *A. caudatus* mainly involved the protein kinases A and C activation, while the effect was partially dependent on the L-type calcium channel and the G protein-coupled exocytosis. *L. mutabilis* effect depended on L-type calcium channels, PKC and PKA systems, G protein-coupled exocytosis and K-ATP channels. The main constituents found in *A. caudatus* were amino acids, sugars, and polyphenols, while *L. mutabilis* extract has higher amounts of alkaloids.

A caudatus is traditionally used to treat disorders in the urinary tract. *L. mutabilis*, on the other hand, is well known for its glycemic lowering effect. Since there is a globally increase of antibiotic resistance, we sought to investigate the possible role of these two medicinal plants in the treatment of urinary tract infections (UTI).

We observed that the hydroethanolic extracts from *A. caudatus* and *L. mutabilis* prevented the uropathogenic *E. coli* (UPEC) *in vitro* infection. *A. caudatus* inhibited the first steps of infection, adhesion, and invasion of UPEC, including ESBL-producing *E. coli* and other uropathogenic bacteria, including multidrug-resistant strains. The effect was attributed to the decrease of gene expression of uroplakin1a and caveolin-1. *L. mutabilis* inhibited UPEC adhesion and interfered in the adhesion process of other Gram-negative and positive uropathogenic bacteria, an effect observed in high glucose concentrations. This effect was explained by a downregulation of gene and protein expression of uroplakin-1a and an upregulation of the antimicrobial peptide RNase 7. Furthermore, the UPEC biofilm formation was inhibited only in low glucose conditions.

In summary, this study provides information about the potential use of *Amaranthus caudatus* and *Lupinus mutabilis*, as a nutraceutical product, since they are part of the traditional Bolivian diet. The hydroethanolic extracts of *Amaranthus caudatus* and *Lupinus mutabilis* have pharmacological properties being promising candidates to treat type 2 diabetes mellitus and to prevent urinary tract infections.

Keywords: Antidiabetic, antibacterial, urinary tract infections, natural products, nutraceuticals, *Amaranthus caudatus*, *Chenopodium quinoa*, *Chenopodium pallidicaule*, *Lupinus mutabilis*, *Smallanthus sonchifolius*, Goto-Kakizaki rats.

LIST OF SCIENTIFIC PAPERS

I. **Silvia Zambrana**, Orlando Mamani, Mabel Canaviri, Maria del Pilar Gutierrez, Sergiu-Bogdan Catrina, Claes-Göran Östenson, Eduardo Gonzales
Glycemia-reducing effects of Bolivian nutraceutical plants
Manuscript

II. **Silvia Zambrana**, Lena C.E. Lundqvist, Virginia Veliz, Sergiu-Bogdan Catrina, Eduardo Gonzales, Claes-Göran Östenson

Amaranthus caudatus Stimulates Insulin Secretion in Goto-Kakizaki Rats, a Model of Diabetes Mellitus Type 2

Nutrients. 2018;10 (1), 94. doi: 10.3390/nu10010094

III. **Silvia Zambrana**, Lena C.E. Lundqvist, Orlando Mamani, Sergiu-Bogdan Catrina, Eduardo Gonzales, Claes-Göran Östenson

Lupinus mutabilis Extract Exerts an Anti-Diabetic Effect by Improving Insulin Release in Type 2 Diabetic Goto-Kakizaki Rats

Nutrients. 2018;10(7), 933. doi: 10.3390/nu10070933

IV. Soumitra Mohanty, **Silvia Zambrana**, Soizic Dieulouard, Witchuda Kamolvit, Vera Nilsén, Eduardo Gonzales, Claes-Göran Östenson and Annelie Brauner

Amaranthus caudatus extract inhibits the invasion of *E. coli* into uroepithelial cells

Journal of Ethnopharmacology. 2018; 28 (220):155-158. doi: 10.1016/j.jep.2018.04.003

V. Witchuda Kamolvit, Vera Nilsén, **Silvia Zambrana**, Soumitra Mohanty, Eduardo Gonzales, Claes-Göran Östenson, and Annelie Brauner

Lupinus mutabilis Edible Beans Protect against Bacterial Infection in Uroepithelial Cells

Evidence Based Complementary Alternative Medicine. 2018; 2018:1098015. doi: 10.1155/2018/1098015

LIST OF SCIENTIFIC PAPERS NOT LISTED IN THESIS

I. Soumitra Mohanty, Witchuda Kamolvit, **Silvia Zambrana**, Corine Sandström, Eduardo Gonzales, Claes-Göran Östenson, Annelie Brauner

Extract of *Clinopodium bolivianum* protects against *E. coli* invasion of uroepithelial cells

Journal of Ethnopharmacology. 2017; 198: 214-220. doi:10.1016/j.jep.2017.01.011

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LIST OF ABBREVIATIONS

A. caudatus	*Amaranthus caudatus*
AGE	Advanced glycation end product
AMPK	Adenosine monophosphate-activated protein kinase
AMPs	Antimicrobial peptides
ANOVA	Analysis of variance
Aq	Aqueous extract
AUC	Area under the curve
C. pallidicaule	*Chenopodium pallidicaule*
C. quinoa	*Chenopodium quinoa* Willd
Cal-C	Calphostin C
cAMP	Cyclic adenosine monophosphate
DAG	Diacylglycerol
DPP-4	Dipeptidyl peptidase 4
Dx	Diazoxide
ESBL	Extended-spectrum β-lactamases
EtOH	Ethanolic extract
EtOH70	Hydroethanolic extract
FBS	Fetal bovine serum
G6P	Glucose-6-phosphate
GC-MS	Gas chromatography-mass spectrometry analysis
GIP	Glucose-dependent insulinotropic peptide
GK rat	Goto-Kakizaki rat
GLP-1	Glucagon-like peptide 1
GLUT-2	Glucose transporter 2
G-proteins	G-coupled receptors
GSH	Reduced glutathione
H-89	N-[2-bromocinnamylamino)ethyl]-5-isoquinoline sulfonamide
HbA1c	Glycated hemoglobin
HBD-1	Human β-defensin-1
HBD-2	Human β-defensin-2
HPLC-HRMS	High performance liquid chromatography-high resolution mass spectrometry analysis
IBCs	Intracellular bacterial communities
IDF	The international diabetes federation
IL	Interleukin

IP3	Inositol 1,4,5-trisphosphate
K-ATP channel	Potassium-sensitive to ATP (K-ATP) channel
KPC-type	*K. Pneumoniae* carbapenemase
KRB	Krebs-Ringer bicarbonate
L. mutabilis	*Lupinus mutabilis*
LPS	Lipopolysaccharide
MDR	Multidrug resistant strains
MIC	Minimum inhibitory concentration
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
NDM-type	New Delhi Metallo-β- lactamase
Nf	Nifedipine
NMR	Nuclear Magnetic Resonance analysis
OGTT	Oral glucose tolerance test
PKA	Protein kinase A
PKC	Protein kinase C
PPARγ	Peroxisome proliferator-activated receptor gamma
Ptx	Pertussis toxin
QIRs	Quiescent intracellular reservoirs
qRT-PCR	Quantitative real-time polymerase chain reaction
RIA	Radioimmunoassay
S. sonchifolius	*Smallanthus sonchifolius*
SGLT2	Sodium–glucose cotransporter-2
STZ	Streptozotocin
T2DM	Type 2 diabetes mellitus
TcpC	Toll/interleukin-1 receptor domain-containing protein
TLR4	Toll-like receptor 4
TZD	Thiazolidinediones
UPEC	Uropathogenic *Escherichia coli*
UPI	Uroplakin
UTI	Urinary tract infections
VDCC	Voltage-dependent Ca2+ channels
VEGF	Vascular endothelial growth factor
W rat	Wistar rat
WHO	World health organization
XTT	(2,3-bis (2-methoxy-4-nitro-5-sulphophenyl)-5-carbox-anilide-2H-tetrazolium, monosodium salt

1 INTRODUCTION

1.1 Pathogenesis of type 2 diabetes mellitus

Type 2 Diabetes Mellitus (T2DM) is a chronic metabolic disorder characterized by elevated glycemia, due to impairment of insulin secretion and/or decreased insulin sensitivity [1, 2]. The latter drives to insulin resistance with reduced glucose uptake in extra-hepatic tissues and augmented endogenous glucose production by the liver [1, 3]. The chronic hyperglycemia [4] and the increased lipolysis together with the elevated free fatty acids [5] may lead to further β-cell impairment that affects the long-term control of glycemia [6] and reduction of peripheral glucose utilization [7, 8]. In the initial stages of the disease, the compensatory insulin secretion by the pancreatic β-cells restores plasma glucose levels, but progressively β-cell function deteriorates, mainly due to inherited β-cell defect(s) [9]. Long exposure to high glucose levels contributes to diabetes-related complications e.g. nephropathy, neuropathy, retinopathy and cardiovascular disease [10, 11] (Figure 1).

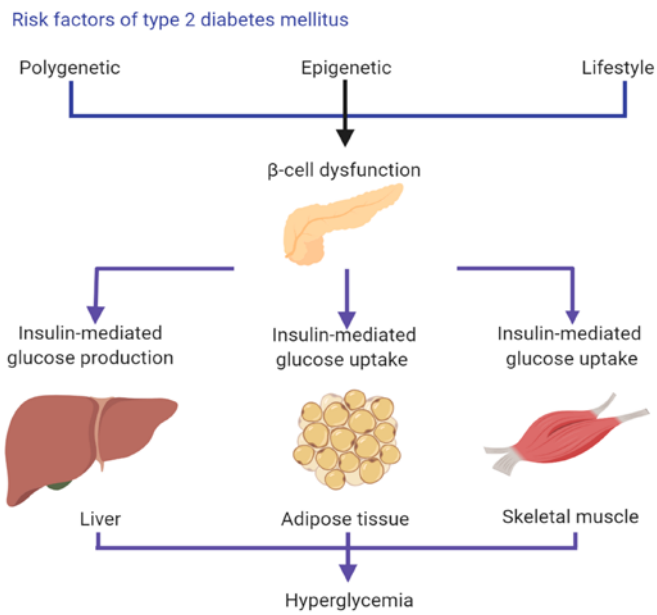

Figure 1. Pathogenesis of Type 2 diabetes mellitus. *Risk factors involved in the pathogenesis of Type 2 diabetes mellitus and effects on pancreas, liver, adipose tissue and muscle.*

1.2 Risk Factors of type 2 diabetes mellitus

T2DM is a result of a complex interaction of polygenetic, epigenetic and lifestyle factors [1, 12]. The lifestyle factors include aging, diet, obesity, lack of physical activity, stress and factors related to urbanization [13]. The high rate consumption of sugar is related to high risk, as well as a diet rich in saturated fats and trans-fatty acids [14]. It has been shown that diet together with physical activity reduce T2DM incidence in individuals with impaired fasting blood glucose [15]. Interestingly, urbanization can lead to psychological stress factors associated with the development of several chronic diseases [16-18].

1.3 Epidemiology of type 2 diabetes mellitus

The prevalence of T2DM, according to the World Health Organization (WHO), in the adult population by 2010 ranged between 7 and 10% and it is estimated that it will be the seventh cause of death by 2030 [19]. T2DM prevalence in developed countries is predicted to increase by 20% between 2010 – 2030, while in developing countries is predicted to increase by 69% [19]. The International Diabetes Federation (IDF) predicts that the number of diabetic patients globally will increase to 693 million by 2045 [20]. T2DM is considered one of the diseases that causes major challenges in the health systems of all countries, regardless of the income level and socioeconomic status. In Latin American countries the mortality among T2DM patients is two times higher compared to healthy individuals [21].

1.4 Insulin secretion pathway

In the β-cells, insulin is released via two major signaling pathways, the potassium-sensitive ATP (K-ATP) dependent-channel and the K-ATP independent-channel pathways. In the K-ATP dependent-channel pathway, glucose enters the β-cells via the glucose transporter 2 (GLUT-2), where it is phosphorylated to glucose-6-phosphate (G6P) by glucokinase enzyme and subsequently metabolized via glycolysis and then oxidized by Krebs cycle to generate ATP.

The ATP leads to an increase in the cytoplasmic adenosine triphosphate/adenosine diphosphate (ATP/ADP) ratio that promotes the closure of K-ATP channels, causing membrane depolarization. The membrane depolarization activates the opening of L-type voltage-dependent Ca^{2+} channels (VDCC), which allows the entry of free extracellular Ca^{2+} into the cells. The resulting increase of the cytoplasmic Ca^{2+} concentration mediates finally the insulin exocytosis [22, 23] (Figure 2).

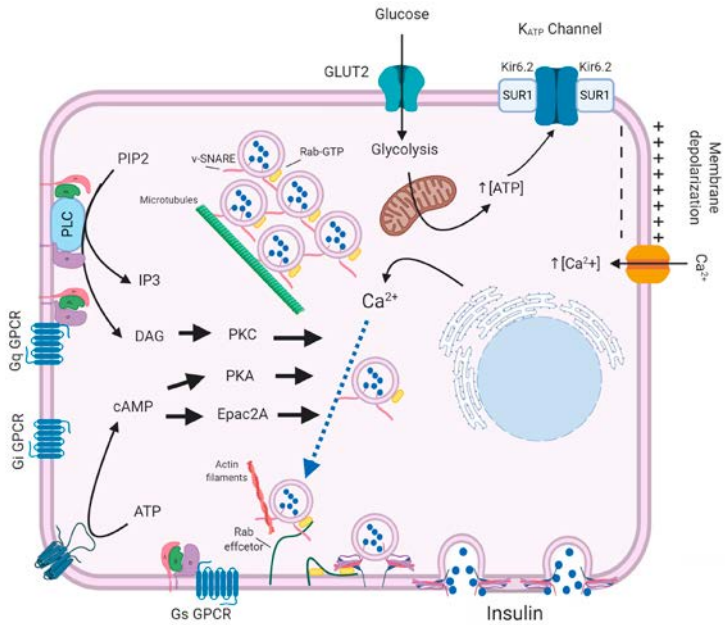

Figure 2. Insulin secretion pathway. *The potassium-sensitive ATP (K-ATP) dependent-channel and the K-ATP independent-channel pathways*

In the K-ATP independent-channel pathway, glucose-induced insulin secretion can be modulated by other cell signals e.g. by hormones such as glucose-dependent insulinotropic peptide (GIP), glucagon-like peptide 1 (GLP-1) and glucagon, which interact with G-coupled receptors (G_s) that transduce the signal by a second messenger, *e.g.* cyclic adenosine monophosphate (cAMP), inositol 1,4,5-trisphosphate (IP3) or diacylglycerol (DAG) [22, 24, 25]. cAMP and DAG increase insulin secretion via protein kinase A (PKA) and protein kinase C (PKC) pathways, respectively. G-protein G_i is coupled to receptors of inhibitory hormones e.g. somatostatin (Figure 2). Besides, the late acting G protein, activated by Ca2+, is involved in the exocytosis of insulin granules [22, 26-28].

1.5 Type 2 diabetes mellitus complications

The chronic hyperglycemia leads to microvascular complications in the retina, renal glomerulus, and peripheral nerve. Diabetic hyperglycemia in combination with dyslipidemia is associated with an accelerated atherosclerotic macrovascular disease that affects arteries that supply the heart, brain and lower extremities with blood. Hence, diabetic patients have a higher risk of myocardial infarction, stroke and limb amputation [12, 29]. Besides, hyperglycemia and insulin resistance might

have an impact on the pathogenesis of macrovascular complications due to the increase of advanced glycation end product (AGE), polyol pathway, activation of PKC isoforms and hexosamine pathway [12, 30].

The polyol pathway converts glucose to fructose by its reduction to sorbitol, which is subsequently oxidized to fructose; sorbitol does not cross cell membrane resulting in intracellular osmotic damage. On the other hand, the activation of the polyol pathway decreases the concentration of pyrimidine nucleotides NADP+ and NAD+. Reduced glutathione (GSH) synthesis requires NADPH, thus a decrease of it would induce oxidative stress [30]. AGEs are the covalent union between a reduced sugar and a free amino group of proteins, lipids, and nucleic acids. Glycation results in altered structure and function of modified biomolecules [30, 31].

Hyperglycemia activates PKC isoforms secondary to ligation to AGE receptors and increase of the polyol pathway. Activation of PKC isoforms mediates abnormalities in retinal and renal blood flow. PKC activation also induces expression of the vascular endothelial growth factor (VEGF) in vascular smooth muscle leading to the pathogenesis of neovascularization and endothelial dysfunction in diabetes [30, 32].

Diabetes-induced glomerular dysfunction such as glomerular hyperfiltration and albuminuria is also mediated by PKC activation [33]. The excess of intracellular glucose goes into the hexosamine pathway and increases levels of uridine diphospho-*N*-acetylglucosamine that further modifies proteins to form *O*-linked glycoproteins [30, 34]. Increased *O*-GlcNAcylation is directly related to hyperglycemia-induced toxicity and is related to other processes *e.g.* redox signaling, apoptosis, autophagy, and protein degradation [35, 36].

1.6 Urinary tract infections

Diabetic patients have a greater frequency of infections often attributed to hyperglycemia, accompanied with impact on the immune system and neutrophil dysfunction interfering with diapedesis and phagocytosis [37]. Especially these patients have increased risk of urinary tract infections (UTI) [38]. UTI are most common in females, mainly involving the bladder (cystitis, bladder infection), but the infection can also ascend and involve the kidneys (acute pyelonephritis, kidney infection) [39, 40]. The urinary tract is sterile, except for the external part of the urethra, due to urine flow, bladder emptying, and defense mechanisms of the epithelia [41].

Women are about four times more susceptible than men to UTI with enhanced risk during the reproductive years [42] and during menopause [43]. Around 50% of women experience at least one episode of UTI during their lifetime with recurrent episodes within 6 months, even after treatment with appropriate antibiotics [40, 42, 44]. Recurrent UTI is defined as two or more episodes within six months or at least three episodes during a year [44]. Therefore, the search for novel approaches for the prevention and treatment of UTIs is needed, [45, 46].

1.7 Uropathogenic *Escherichia coli*

Approximately 90% of all UTIs are caused by uropathogenic *Escherichia coli* (UPEC) [47-49]. UPEC has several virulence factors that play a role in the infection *e.g.* lipopolysaccharide (LPS), toll/interleukin-1 receptor domain-containing protein (TcpC), α-hemolysin, siderophores, adhesive organelles pili or fimbriae type 1, P, S, and F1C pili, and capsule [50]. Type 1 fimbriae possess four subunits FimA, FimF, FimG and FimH and a short flexible tip composed of minor pilins [51].

1.8 Pathogenesis of urinary tract infections

UTIs result from bacterial transfer from the intestine via the perineum to the urinary tract, where they can colonize and cause infection [52]. The urothelium is a transitional epithelium with a basal layer of stem cells, consisting of one or more intermediate layers and the superficial layer, with highly differentiated, multinucleated superficial facet cells known as umbrella cells. The human bladder urothelium supports frequent changes in volume, thus the umbrella cells have a particular composition of lipids and proteins that confers permeability and regulates the passage of water, ions and large macromolecules [44, 53]. The apical membrane has four integral membrane proteins the uroplakins (UPIa, UPIb, UPII, and UPIII) that cover the luminal surface of the bladder [54].

Urothelial plaques are rich in cholesterol and sphingolipids, molecules that favor a microdomain formation, named lipid rafts, structures that lower membrane fluidity and permeability. Lipid rafts have also glycolipids and glycosylphosphatidylinositol-anchored molecules and might contain caveolin-1 proteins, that form a subtype of lipid rafts, the caveolae. Lipid rafts are implicated in signal transduction, they also possess a versatile endocytic capacity. *In vitro* binding assays have shown that UPEC expressing type 1 pili specifically binds to UPIa and UPIb, located in lipid rafts [54-56]. FimH mediates specific recognition of UPIs and bacterial adhesion, which is the first step of bacterial infection and may lead to invasion that occurs via a cholesterol and dynamin-dependent phagocytosis, process modulated by calcium levels, and clathrin adaptor AP-2 that recognize NPXY motifs of the cytosolic tail of β1 integrin [57, 58] (Figure 3).

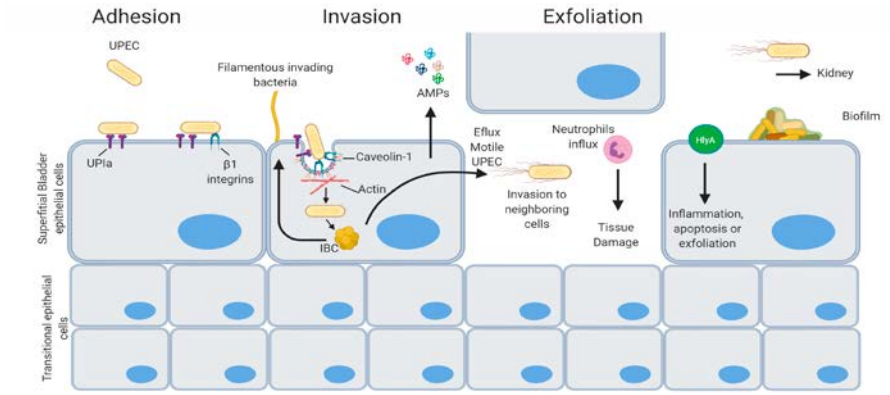

Figure 3. Pathogenesis of uropathogenic E. coli infection. *The stages of UPEC infection are shown; adhesion, invasion and exfoliation. UPEC adheres to uroepithelium through FimH from type 1 pili fimbriae, binding the receptors UPIa. Invasion is mediated by β1-integrins. Internalized bacteria can form IBCs. Once UPEC have attached, AMPs synthesis increases. HlyA toxin triggers signals of inflammation, apoptosis or exfoliation. Filamentous UPEC flux out and invade the surrounding superficial cells. From IBC, motile UPEC efflux and are able to invade neighboring cells. UPEC also form biofilms on superficial cells. Motile UPEC can ascend to the kidney.*

It was observed that in pyelonephritic strains, the type 1 fimbriae expression decreases to allow UPEC ascending to the kidneys, where they attach to digalactoside receptors by P fimbriae. FimH can also interact with UPIIIa and stimulate signaling pathways for invasion and apoptosis [50, 52]. Invasion mediated by FimH interacts with α3 and β1-integrins, clustered with actin and destabilized microtubules [52, 54, 57]. The local actin rearrangement results in the internalization of bacteria; once internalized, UPEC reside within Rab27b/CD63/Caveolin-1-positive fusiform vesicles and evades expulsion and phagolysosomal death replicating rapidly into the cytoplasm to form biofilm-like complexes both extra and intracellularly, the intracellular bacterial communities (IBCs) and located deeper in the tissue also forming quiescent intracellular reservoirs (QIRs) which serve as transient, protective environments [50, 59, 60].

IBCs formation requires continued type 1 pilus expression after the invasion and produces structural components, such as antigen 43, a polysaccharide-rich matrix and capsular proteins [59]. Dispersal of UPEC from the IBC is a crucial event for bacterial persistence. Inside the IBCs, bacteria are coccoidal, but have a filamentous form when they emerge from the dying cells and may then colonize and invade neighboring cells [61].

IBCs are important in UTI pathogenesis and urine sediments from women with recurrent UTI have been demonstrated to contain exfoliated bladder epithelial cells with IBCs and higher numbers of IBC during acute infection are associated with the development of chronic cystitis [44, 59].

During infection, bacterial adhesion stimulates the immune system with the production of antimicrobial peptides and cytokines/chemokines and recruiting neutrophils that later may induce tissue damage. Further, the invasion that follows the adhesion ends up in apoptosis and exfoliation of bladder cells [39, 53, 62].

1.9 Biofilm

Biofilm is a bacterial consortium structured within a polymer matrix of polysaccharide, protein and extracellular DNA [61]. The bacterial biofilm is a complex structure, which makes it difficult for antibiotics to penetrate, and also makes difficult for the innate and adaptive immune response to reach the bacteria [61, 63]. The bacterial transition from planktonic to biofilm growth facilitates bacterial survival since, in the matrix, nutrients are trapped for metabolic utilization and water is efficiently retained. Bacteria become adapted due to changes in gene expression and they can acquire genes for antibiotic resistance from resistant clones that could be present inside the biofilm [61, 64, 65].

1.10 Immune response in urinary tract infections

The urinary tract is a sterile environment, the urine flow, and micturition rinse away nonattached or weakly adherent microbes. The characteristics of urine, low pH and osmolarity, salts, urea, and organic acids inhibit bacterial growth [53]. The local microbiota normally present in the urogenital tract serve as another origin of defense by altering the pH and producing their antimicrobial products, which may help controlling infection [43, 66]. Other molecules as soluble and cell-associated factors as uromodulin, a renal-specific protein secreted in the urine and secretory IgA, act as anti-adherence factors [55, 67].

The type 1 fimbriae stimulate cytokine and chemokine synthesis and trigger exfoliation of superficial uroepithelium [62]. Exfoliation of infected bladder epithelium is a host defense mechanism in patients with UTIs. Bladder cell exfoliation is an apoptosis-like mechanism involving DNA fragmentation and caspases activation [68]. Neutrophil recruitment is critical for bacterial clearance and neutrophil accumulation is observed in the urine as pyuria, which is an indicator of UTIs. Bladder epithelial cells induce the expression of ICAM-1 a factor for neutrophil migration [67].

During UTI proinflammatory cytokines like IL-1, IL-6 and IL-8 are increased. IL-6 levels relate to the severity of UTI, and together with IL-8, a strong chemoattractant, correlate with the recruitment of neutrophils in urine [50, 62]. Cytokine production in kidney epithelial cell is produced partly due to the interaction with P-pili, through a ceramide and serine-threonine kinase-dependent signaling pathway [39, 55]. Toll-like receptor 4 (TLR4), the major LPS sensor in mammals, is present on the luminal surface of the bladder epithelium. CD14, LPS co-receptor, is required for TLR4 activation, is present in low levels in healthy kidney; after microbial attachment, epithelia liberates endogenous ceramides that trigger TLR4-dependent responses and IL-8 [49, 50, 62].

The inflammasome NLRP3 plays a role in the progression of UTI. It detects extra- and intracellular bacteria, which activate the caspase-1-dependent cell dead (pyroptosis), or induce the release of pro-inflammatory cytokines, e.g. IL-1β and IL-18 [39, 62]. The inflammasome activation and pyroptosis by UPEC contribute to inhibition of intracellular bacterial of replication niches [39].

1.11 Antimicrobial Peptides

Antimicrobial peptides (AMPs) are oligopeptides found in humans, animals, and plants. They are produced by the lymphatic system, gastrointestinal tract, genitourinary tract, and immune system [69] and confer instant protection against pathogens and modulate the immune system [48, 70] against a wide variety of microorganism including cancerous cells [71, 72]. AMPs have advantages over conventionally used antibiotics, they have broad-spectrum activity with rapid bacterial killing and although they have been known for long, only little resistance is known [73].

AMPs are positively charged and most of them are amphipathic polycationic peptides, because of their content of lysine and/or arginine amino acids that primarily target the negatively charged bacterial cell membrane. Seconds after the initial contact, the disintegration of the lipid bilayer structure is caused [53, 69, 72-74].

Additionally, AMPs have immunomodulatory effects including chemotactic attraction, chemokine induction, histamine release, angiogenesis, wound healing, or adaptive immunity [71]. In mammals, AMPs modulate the immune system by activation of T cells, activation via Toll-like receptors, increase of phagocytosis, activation of dendritic cells, and chemoattraction of neutrophils [71, 72]. Macrophages, neutrophils and epithelial cells produce AMPs constitutively [71].

1.12 Antimicrobial Peptides in human urinary tract infections

Among the human AMPs, defensins and cathelicidins are present in the urinary tract [74]. Human β-defensin-1 (HBD-1) is constitutively expressed in bladder epithelial cells and highly expressed in the kidney [48, 53]. The luminal surface of the tubular epithelia contains higher anti-infective concentrations of HBD1 [74]. β-defensin-2 (HBD-2) is induced in response to inflammatory stimuli during UTI and has been detected in chronically-infected kidneys [48]. LPS induces transcription of HBD-2 and a slight increase of HBD-1 [75].

Cathelicidin, also called LL-37, is produced in the kidney tubular cells as well as urothelial cells [74]. AMPs in healthy urine do not reach microbicidal or inhibitory concentrations, an important fact that may prevent the development of resistance to the AMPs [76]. LL-37 is constitutively expressed in the urinary tract and after bacterial interaction levels of mRNA increase and within minutes, peptide secretion is detected in the surrounding region [77]. LL-37 functions as neutrophils and mast cells chemoattractant, prevents apoptosis in neutrophils and keratinocytes, promotes angiogenesis, monocyte differentiation, vascular endothelium proliferation, and exhibits anti-inflammatory and anti-endotoxic effects [71].

RNase 7 is produced by the bladder urothelium, secreted into urine and produced in the intercalated cells of the collecting tubules of the kidney [78]. It induces local membrane destabilization due to its affinity for LPS from Gram-negative bacteria and peptidoglycan from both Gram-negative and Gram-positive bacteria [78]. RNase 7 has broad-spectrum microbicidal activity against many common uropathogens [67, 79, 80]. When RNase7 is neutralized in human urine specimens, bacterial growth increases [81].

AMPs are active against biofilm-forming bacteria, not only for their capacity to access bacteria in biofilms but they may also prevent biofilm formation. While higher concentrations of LL-37 are needed to kill bacteria, subinhibitory concentration can prevent the formation of new biofilm [82]. KT2 and RT2 modified AMPs, the semi-synthetic peptide SB056, peptide 1018 and others have been constructed and shown to be active against both planktonic and biofilm-forming bacteria [73]. On the contrary, different mechanisms of AMP resistance by biofilm producing bacteria have been described due differences in motility or metabolism of the bacterial subpopulations [73, 83].

1.13 Diabetes and urinary tract infections

The prevalence and severity of UTI increase in diabetic patients [37, 84] since they are prone to UTI-related complications due to conformational and molecular changes in bladder epithelium and disruption of the host immune response [85-87]. Predisposition to UTIs in T2DM results from factors as glycosuria; increase bacterial adherence and immune dysfunction. T2DM patients treated with the sodium-glucose cotransporter-2 (SGLT2) inhibitors tend to develop UTI by the induced glycosuria [86].

Bacterial adhesion, mediated by UPEC type 1 fimbriae, in diabetic patients is increased due to changes in the carbohydrate moieties in the glycoproteins of the luminal urothelial surface [85, 88]. During diabetes progression, the accumulation of AGEs, enhances bacterial adhesion, which has been proved by *in vitro* binding of type 1 fimbriae to several AGE products and further confirmed in diabetic mice where treatment with AGE inhibitor pyridoxamine reduces bacterial adhesion [85, 88].

1.14 Current therapy of type 2 diabetes mellitus

The antidiabetic therapy goal is to achieve optimal glycemic control, and thereby to prevent or delay the progression of complications [89]. Ideally, the antidiabetic therapy should also help the patient to avoid episodes of pronounced hyper or hypoglycemia. Current antidiabetic medication includes biguanides, sulfonylureas, meglitinides, thiazolidinediones (TZD), dipeptidyl peptidase 4 (DPP-4) inhibitors, GLP-1 analogs (also called incretins), sodium-glucose cotransporter (SGLT2) inhibitors, and α-glucosidase inhibitors [89, 90].

The sulfonylureas increase insulin secretion by blocking the K-ATP channels in pancreatic β cells. The first-generation sulfonylureas (e.g. chlorpropamide, tolazamide, and tolbutamide) have a long half-life, higher risk of hypoglycemia, and slower onset of action, as compared to the second-generation (e.g. glipizide, glimepiride, and glibenclamide) [91, 92].

Meglitinides *e.g.* repaglinide and nateglinide, are non-sulfonylurea secretagogues, that bind to the sulfonylurea receptor in β-cells, however weaker than sulfonylurea, leading to a short-acting insulin-releasing effect [93].

Metformin is a biguanide that activates the adenosine monophosphate-activated protein kinase (AMPK) in the liver to induce hepatic glucose uptake and to inhibit gluconeogenesis. Besides, the substance has a weak effect on glucose uptake in skeletal muscle by AMPK activation. Metformin confers a low risk of hypoglycemia and low probabilities of weight gain [94].

The incretins exert antidiabetic effects by several mechanisms, i.e. stimulation of the glucose-dependent insulin secretion, suppression of glucagon secretion, slowing down the gastric emptying, and reduction of appetite, thereby also inducing weight loss. In T2DM patients, the incretin effect of the main endogenous incretin, glucagon-like peptide (GLP-1) is usually reduced [95]. The incretin mimetics include two types of drugs, GLP-1 receptor agonists and DPP-4 inhibitors. Current GLP-1 receptor agonists are e.g. exenatide and liraglutide. Dipeptidyl peptidase 4 (DPP-4) inhibitors include sitagliptin, saxagliptin, vildagliptin, linagliptin, and alogliptin [96]. Their mode of action is to increase the endogenous GLP-1 by inhibiting the degradation of the hormone by the DPP-4 enzyme [90, 97].

The sodium-glucose co-transporter inhibitors (SGLT2 inhibitors) lower blood glucose levels by blocking glucose reabsorption from the primary urine in the proximal renal tubule. Available SGLT2 inhibitors are canagliflozin, dapagliflozin, and empagliflozin. These drugs have also been shown to reduce the risk of cardiovascular diseases [90, 97, 98].

Rosiglitazone and pioglitazone are thiazolidinedione (TZD) agents and agonists of the peroxisome proliferator-activated receptor-gamma (PPARγ). TZDs facilitate glucose uptake in adipose tissue, muscle, and liver; reduce free fatty acid accumulation and inflammatory cytokines; augment adiponectin, and preserve β-cell integrity and function [89, 90, 97].

The alpha-glucosidase inhibitors compete and reversibly inhibit the intestinal alpha-glucosidases. Acarbose prolongs the carbohydrate digestion time thus, reduces the rate of glucose absorption [99].

Insulin is often applied subcutaneously when monotherapy or a combination of oral antidiabetic drugs fail. *Rapid-acting insulin analogs (lispro, aspart, or glulisine)* are used before meals, often in combination with long-acting, basal insulin *(NPH, detemir, glargine, or degludec)* once or twice daily [100]. To control the peripran-dial glucose *premixed (biphasic) insulin analogs (70/30 aspart mix, 75/25 or 50/50 lispro mix*) can be administrated two or three times [101, 102].

1.15 Current therapy against urinary tract infections

Treatment guidelines for uncomplicated UTI recommend the use of nitrofuran-toin and pivmecillinam as first choice, while trimethoprim and cefadroxil are the second choices [43, 103, 104]. In specific cases, antibiotic prophylaxis can be used during a prolonged, but limited period [48]. However, the increase in antimicrobial resistance makes treatment strategies more complex [105].

The high frequency of extended-spectrum β-lactamases (ESBL) *E. coli* or *K. pneumoniae,* which inactivate β- lactam antibiotics such as cephalosporins [106] has extended the use of carbapenems. Carbapenem resistance, $ESBL_{CARBA}$, is the result of the production of carbapenemases, e.g. *K. pneumoniae* carbapenemase (KPC-type), New Delhi Metallo-β- lactamase (NDM-type) and OXA-48-type enzymes. Unfortunately, worldwide consumption of carbapenems, especially in developing countries, increased the carbapenem resistance.

The acquisition of ESBL or carbapenemase genes is mediated by the transference of resistance gene carried in plasmid, resulting in the development of multidrug-resistant strains (MDR); strains that are resistant to 3 groups of antibiotics [106, 107].

1.16 Medicinal plants in the treatment of type 2 diabetes mellitus

Natural products are believed to be safe and some T2DM patients use them to avoid the progression of the disease or its complications [108, 109]. More than 1200 plant species and many natural products, vitamins, and minerals are reported to be used as complementary or supplement treatment in T2DM [110, 111].

Momordica charantia, aerial parts and seeds, have insulin mimetic and insulin secretagogue activity in animal models and in clinical trials [109]. *Cinnamomum verum* (cinnamon) reduces the fasting plasma glucose, low-density lipoprotein-C, triglycerides, and total cholesterol [112, 113]. *Morus alba* reduces postprandial blood glucose in women with impaired glucose tolerance [114]. *Zingiber officinale* Roscoe (ginger) enhances the insulin secretion and glucose uptake of in skeletal muscles [115]. Ginger as a dietary supplement reduces the glycated hemoglobin (HbA1c) [113, 116]. *Gymnema sylvestre* regenerates pancreatic islet and inhibits the glucose absorption from the intestine [115]. *Gynostemma penthaphyllum* contains substances that increase insulin sensitivity [117] and insulin secretion [118, 119] and also suppresses the hepatic glucose output [120].

Among the phytochemical compounds reported to have antidiabetic properties, we can mention terpenoids, flavonoids, anthocyanins, and alkaloids. Triterpenoids improve glucose uptake and insulin secretion. The flavonoid naringenin reduces the intestinal glucose absorption, reduces the renal glucose reabsorption, and increases glucose uptake in muscle and fat tissues. Kaempferol has an antioxidant effect that reduces the oxidative stress and preserves the pancreatic β-cell mass [121]. Anthocyanins from blueberries improve regulation of the glucose uptake in skeletal muscle, decrease the glucose production in the liver and protect the β-cells function due to their antioxidant properties [122]. Alkaloids e.g. berberine, lupanine neferin, and piperine improve the insulin-signaling pathway cascades in β cells, myocytes, adipocytes, and hepatocytes [121].

1.17 Medicinal plants in The treatment of urinary tract infections

The traditional medicine in different countries use different plant preparation to treat UTI, some of them have scientific evidence that supports their use [111, 123]. Some plant extracts exhibit direct antibacterial effect against uropathogenic bacteria [124, 125], others interfere with bacterial adhesion by interaction with bacterial outer membrane proteins as reported for rhizome of *Agropyron repens* L. and the stigmata of *Zea mays* L. [126]. Cranberry components interfere with bacterial adhesion by direct interaction with P-fimbriae of UPEC [127, 128]. However, in a clinical study, patients with recurrent UTI were either given 12 months prophylaxes with cranberries or trimethoprim-sulfamethoxazole. In spite of the *in vitro* results, patients on cranberry prophylaxes had more recurrences, which occurred earlier than those on trimethoprim-sulfamethoxazole [129].

The combination of plant extracts plus D-mannose, a prophylactic agent that inhibits bacterial adherence binding and blocking FimH adhesin [130], prevents the recurrence of UTIs. Patients treated with berberine, arbutin, birch, and forskolin and D-mannose had a lower incidence of cystitis [131].

Furthermore, extracts from medicinal plants have also demonstrated an effect on the host cell, inhibiting in vitro UPEC adhesion as reported for *Orthosiphon stamineus* and *Urtica spp*. [126]; and Luteolin-glycosides and related flavons from *Apium graveolens* fruits [132]. In previous studies we have shown that *Clinopodium bolivianum in vitro* decreases UPIa expression, resulting in lower adhesion and invasion of UPEC and up-regulates caveolin-1. In uninfected cells, however, IL-8 decreased compared to non-treated uroepthelial cells. Moreover, *Clinopodium bolivianum* reduces UPEC biofilm formation [133]. *Citrus reticulata* decreased β1-integrin expression and reduced bacterial invasion [134].

Inhibitory effect of biofilm has been reported by *R. officinalis* and *Origanum majorana oil* [124] and extracts from *Herniaria glabra* and *Vaccinium vitisidaea* [135].

1.18 Nutraceuticals source of Novel Therapies

Natural products are a potential source of novel therapies; most of them are safe and are an attractive complement to the regular therapies [111, 115]. Among natural products a nutraceutical is “a food or part of a food that provides health benefits in addition to its nutritional content” [136, 137]. Nutraceuticals are well accepted in human consumption, being food with pharmaceutical effect. However, in most cases, evidence of the pharmacological properties is moderately explained or absent [138, 139]. Thus, it would be important to understand the mechanisms of action of active substances contained in nutraceuticals that may improve health or prevent pathologies [140].

Different natural products with antidiabetic and/or antibacterial properties have been reported to improve glycemic control and prevent long-term complications [141, 142]. Interestingly, Bolivia has huge biodiversity that could be a potential source of new treatment drugs. Thus, the alternative approach proposed by this study is to evaluate the antidiabetic and antibacterial promising plants, which will be a source of potential nutraceutical products [111, 143, 144].

1.19 Plants under study

Based on their traditional use, we have selected five Bolivian food plants with potential nutraceutical effects; the following information highlights Andean plants that are currently part of the Bolivian nutrition schemes [111, 144].

Amaranthus caudatus (*A. caudatus*), common name kiwicha, is a pseudocereal, its seeds are usually consumed in beverages, food preparations e.g. soup, bread or as toasted flour. Traditional medicinal properties attributed to *A. caudatus* are blood purifier, diuretic, and digestive [145-148]. Among its pharmacological properties, it showed anti-hyperglycemic activity, and studies using water decoction extract reported α-amylase inhibitory activity [149]. In addition, the methanolic extract of *A. caudatus* leaves reduced glycemia and lipid profile of streptozotocin (STZ)-injected rats, a model of type I diabetes [147] (Figure 4).

Figure 4. Pseudocereal plants under study. *Photographs of plants and seeds of Amaranthus caudatus (A-B) Chenopodium quinoa Willd (C-D), Chenopodium pallidicaule (E-F), respectively.*

Chenopodium quinoa Willd (*C. quinoa*), common name quinua, is a pseudo-cereal that has a balance between oil, protein, and fat and it is considered as a nutraceutical that may lower the risk of various diseases due to its content of minerals, vitamins, and antioxidants [148]. *C. quinoa* seeds are also used in food preparations as part of the diet of rural Bolivian families, including a variety of soups and refreshments [150]. Phenolic compounds responsible for the antioxidant activity and anti-radical, reducing power, represent the nutraceutical potential of *C. quinoa* [151-153] (Figure 4).

Chenopodium pallidicaule (*C. pallidicaule*), common name kañiwa, is a pseudo-cereal, its seeds are usually consumed in refreshments [153]. *C. pallidicaule* anti-oxidant properties and composition are similar to that of *C. quinoa* and *A. caudatus* [154-156]. *C. pallidicaule* contains a variety of functional molecules; flavonol glycoside, quercetin, isorhamnetin, and kaempferol, components identified in its raw whole seed flour. Their quercetin derivatives were correlated with their high radical scavenging-linked anti-oxidant activity [157, 158] (Figure 4).

Lupinus mutabilis (*L. mutabilis*), common name tarwi, is a legume that contains proteins rich in the essential amino acid lysine [159] (Figure 5). Its seeds contain high amounts of alkaloids, molecules responsible for its bitter taste [160]. Ethanolic extract of *L. mutabilis* showed hypoglycemic activity in normoglycemic rats with 49% of reduction of glycemia. Furthermore, a clinical trial showed that *L. mutabilis* consumption in normal healthy young individuals did not change importantly the glycemia and insulin, but, similar doses significantly reduce glycemia in hyperglycemic individuals [161].

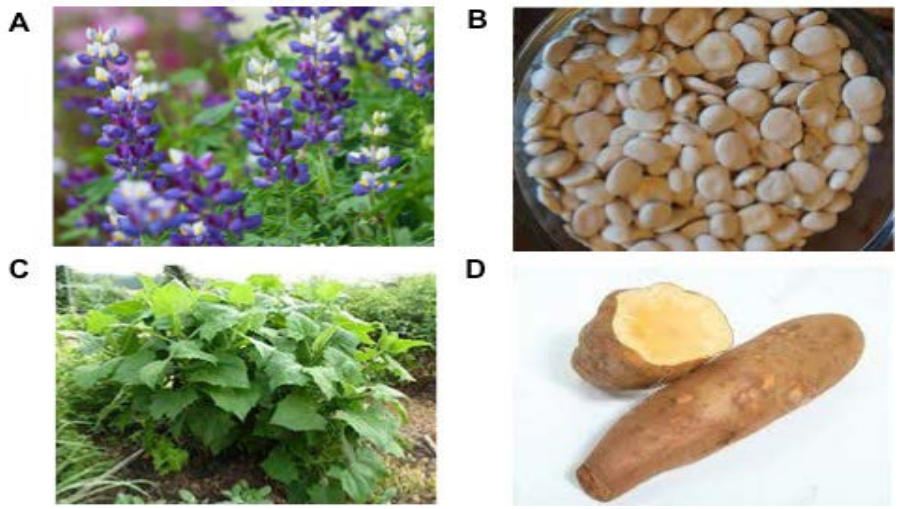

Figure 5. Edible bean and tuberous root under study. *Photographs of Lupinus mutabilis, plants and seeds (A-B) and Smallanthus sonchifolius, plant and tuberous roots (C-D)*

Smallanthus sonchifolius (*S. sonchifolius*), common name yacon is a tuberous root consumed in rural valley regions where it is well known for its property to induce weight loss [162]. Its content of inulin and fructo-oligosacharides, components that cannot be digested, makes it a hypocaloric food ideal for consumption in diabetic patients [163]. Daily administration of organic extracts from *S. sonchifolius* leaves for 8 weeks increases the plasma insulin levels [164]. The water extract of its leaves reduced glycemia, as well as body weight in type I diabetic STZ rats [165] and its decoction significantly decreases glycemia in STZ-diabetic rats by the inhibition of the α-glucosidase [166]. *S. sonchifolius* roots flour as a diet supplement increases the glutathione peroxidase and glutathione levels in the liver and kidney in diabetic rats [167] (figure 5).

2 AIMS

The overall objective of this thesis was to evaluate the potential antidiabetic and antibacterial effect of selected Bolivian nutraceutical plants, traditionally used to prevent such diseases.

Specific aims were:

1. To screen the glycemia-reducing effect of aqueous, ethanolic and hydroethanolic extracts from five Bolivian nutraceutical plants e.g. *Amaranthus caudatus*, *Chenopodium quinoa*, *Chenopodium pallidicaule*, *Lupinus mutabilis* and *Smallanthus sonchifolius* using healthy Swiss albino mice.

2. To evaluate the putative antidiabetic effect and the mechanism of action of *Amaranthus caudatus* and *Lupinus mutabilis* in the type 2 diabetic model the Goto-Kakizaki rats and the healthy control, Wistar rats.

3. To evaluate the antibacterial effect of *Amaranthus caudatus* and *Lupinus mutabilis* using *in vitro* infection with uropathogenic *E. coli* and other uropathogenic bacterial strains in uroepithelial cells.

3 MATERIAL AND METHODS

3.1 Experimental design

The present study explored the antidiabetic and antibacterial properties of Bolivian plants under the following experimental approach (Figure 6).

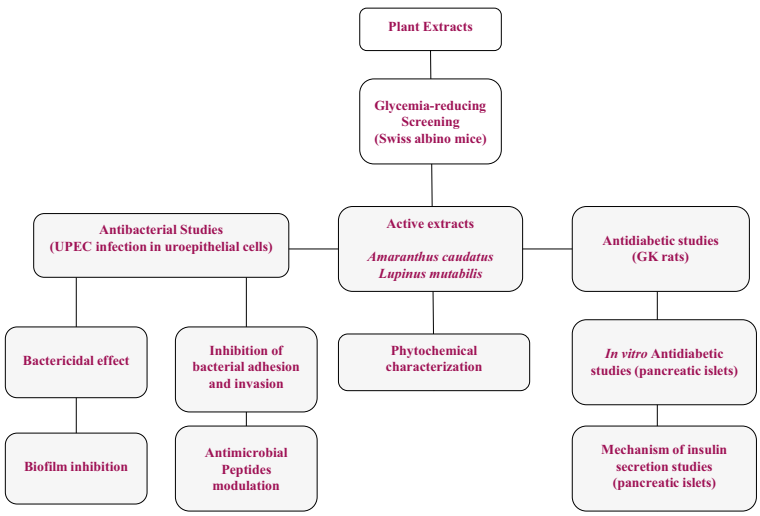

Figure 6. Experimental design followed in antidiabetic and antibacterial studies.

3.2 Plant extracts and phytochemical analysis

The plant specimens were collected from local producers to have locally produced material avoiding possible contamination with other plant species or possible contamination during storage and selling. Location was recorded to have that reference for further collections. All plant species were prepared for authentication to verify the correct taxonomy assignation by the Herbario Nacional de Bolivia [168] (Table 1) **(Paper I, II, III, IV and V)**.

Table 1. Information of collection places of plants under study

Scientific name	Location	Coordinates
Amaranthus caudatus *(A. caudatus)*	Tomina, Tomina Province, Chuquisaca	19°25´53.96´´S 64°15´5.44´´W
Lupinus mutabilis *(L. mutabilis)*	Ancoraimes, Omasuyos Province, La Paz	15°55´19.3´´S 68°53´50.1´´W
Chenopodium quinoa *(C. quinoa)*	Huancane, Los Andes Province, La Paz	16°18´14.14´´S 68°32´35.88´´W
Chenopodium pallidicaule *(C. pallidicaule)*	Peñas, Los Andes Province, La Paz	16°13´55.02´´S 68°29´41.70´´W
Smallanthus sonchifolius *(S. sonchifolius)*	Sorata, Laracaja Province, La Paz	15°45´12.18´´S 68°39´19.46´´W

Plant extracts were prepared using different solvents, distilled water (aqueous extract, Aq), 70% ethanol solution in distilled water (hydroethanolic extract, EtOH70) and absolute ethanol (ethanolic extract, EtOH). Solvents were removed using a rotary evaporator and a freeze drier [133, 169] **(Paper I, II, III, IV and V)**.

Initially, the hydroethanolic extracts were characterized using standard phytochemical qualitative tests [170] **(Paper I)**. In further studies, active hydroethanolic extracts were analyzed by High Performance Liquid Chromatography-High Resolution Mass Spectrometry Analysis (HPLC-HRMS) followed by Nuclear Magnetic Resonance analysis (NMR) for *A. caudatus* and Gas Chromatography-Mass Spectrometry Analysis (GC-MS) for *L. mutabilis*. Chemical standards were used to assign tentative identification [133] **(Paper II, III, IV and V)**.

3.3 Antidiabetic Studies

3.3.1 Swiss albino mice

Swiss Albino mice were used in screening for glycemia-reducing effect and in acute toxicity studies. This mouse is an outbred strain, with some variability between animals. Despite its variability, following the standard protocols [171], the parameters we measured, hematological, blood biochemical parameters and behavior were enough reproducible. Animals were provided by the animal facility of the Facultad de Ciencias Farmacéuticas y Bioquímicas, Universidad Mayor de San Andrés **(Paper I)**.

3.3.2 Goto-Kakizaki rat model

The Goto-Kakizaki rat (GK rat), a non-obese model of T2DM, was used in antidiabetic studies. This model was originated by repeated inbreeding of Wistar (W) rats with impaired glucose tolerance and is characterized by impaired β-cell mass and function and glucose intolerance [9]. Constant features of GK rat are glucose intolerance, mainly due to impaired glucose-induced insulin secretion. In GK rat islets, the GLUT2 is under-expressed but the islet glycolytic rates are normal or increased. One major defect in GK rats is the reduced islet expression of the exocytotic soluble SNARE receptor proteins involved in the docking and fusion of insulin granules [172]. Additionally, PKC isoenzymes in GK rat islets are diminished and/or have abnormal activation [9]. GK rats used came from the Stockholm colony bred in the animal facilities of the Department of Molecular Medicine and Surgery, of the Karolinska Institutet [173, 174]. W rats, used as healthy control animals were purchased from Charles River, a commercial breeder.

3.3.3 Ethical considerations

Glycemia reducing screening studies, performed in Bolivia, were approved by the Ethics committee from the Universidad Mayor de San Andrés; approval CEI-UMSA 0115 **(Paper I)**. Anti-diabetic studies, performed in Sweden, were approved by the Laboratory Animal Ethics Committee of the Karolinska Institutet; approval Dnr. N50/2014 **(Papers II and III)**.

3.3.4 Screening for glycemia-reducing effects

The effect of plant extracts on glycemia was evaluated in non-fasted mice by single oral administration. Extract doses tested were: *A. caudatus, L. mutabilis, C. quinoa*, and *C. palidicaule*, ethanolic extract (4000 and 2000 mg/kg b.w.), hydroethanolic and aqueous extracts (2000, 1000 and 500 mg/kg b.w). *S. sonchifolius,* ethanolic and aqueous extracts (1000 mg/kg b.w.), and hydroethanolic extract (1000, 500 and 250 mg/kg b.w). For experimentation, aqueous and hydroethanolic extracts were dissolved in distilled water, and ethanolic extracts were dissolved in 1.5% water solution of Tween 20. The placebo groups received distilled water and 1.5 % water solution of Tween 20. Blood samples from the tip of the tail were collected 1, 2, 4 and 6 h, after administration of the extracts [175]. Glycemia was measured using a glucometer **(Paper I)**.

3.3.5 Oral Glucose Tolerance Test

The oral glucose tolerance test (OGTT) was used to evaluate the clearance of an oral glucose load in fasted animals; in parallel, the glucose-dependent insulin secretion can be followed [176, 177]. Extracts were orally administrated, one hour

before the test. After the glucose challenge, (3 g/kg b.w.) for Swiss mice and W rats, while (2 g/kg b.w.) for GK rats, blood samples were collected in intervals of time to follow the glucose clearance (0, 15, 30, 60, and 120 min). Blood samples were collected after cutting tip of the tail, to get enough sample to detect glycemia by using a glucometer [118, 173, 174]. Serum insulin was also measured at time 0 and 30 min, determined by radioimmunoassay (RIA) [178]. The improvement in glucose tolerance was analyzed at each time point compared with placebo controls and also by calculating the area under the glucose clearance curves (AUCs) [179] **(Papers I, II and III)**.

3.3.6 Long-term Treatment Evaluation

The effects of *A. caudatus* and *L. mutabilis* were evaluated in long-term oral treatment of GK and W rats during 21 days, according to the following scheme (Figure 7) **(Papers II and III)**:

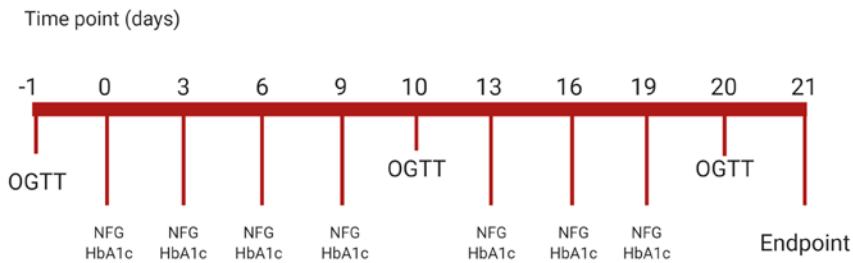

Figure 7. Experimental approach followed in long-term experiments. *Treatment during 21 days group 1: GK rats treated with A. caudatus or L. mutabilis (1000 mg/kg b.w.); group 2: GK rats, placebo vehicle; group 3: W rats treated with A. caudatus or L. mutabilis (1000 mg/kg b.w.); group 4: W rats, placebo vehicle. Time intervals for sampling collection are indicated to conduct the OGTT, non-fasting glucose (NFG), glycated hemoglobin (Hb1Ac), and islets collection at the endpoint of treatment.*

3.3.7 Sub-acute toxicity studies

The sub-acute toxicity of extracts was evaluated in W rats. Dry extracts were incorporated to regular chow food in quantities to reach a daily dose of 1000 mg/kg b.w. of *A. caudatus* or *L. mutabilis*. Treatment was conducted for 28 days, following changes in behavior and weight; at the end point blood sample was collected to determine hematological and biochemical parameters, according to guideline 407 of the Organization for Economic Cooperation and Development [180] (**Papers II and III**).

3.3.8 Pancreatic Islets Isolation

To explore the effect of *A. caudatus* and *L. mutabilis* on β-cell function, we measured the insulin release in isolated pancreatic islets. To digest the pancreas in the isolation procedure we used collagenase type I dissolved in HBSS 9 mg/10 ml for Swiss mice **(Paper I)** and Wistar rats **(Papers II and III)**, and 24 mg/10 ml for GK rats **(Papers II and III)**, followed by a gradient density isolation of islets using a mixture of Histopaque 1077 and 1119, ratio 2:1. The free islets were hand-picked using a stereomicroscope and were then stabilized by culturing them overnight in RPMI supplemented with 30 mg of L-glutamine, antibiotics (100 UI/ml penicillin and 0.1 mg/ml streptomycin) and 10% of heat-inactivated fetal calf serum [174, 181].

3.3.9 Insulin Release

Overnight-cultured round shape and well-defined islets were collected in batches of 3 islets per condition. After a preincubation during 30 min in KRB (Krebs-Ringer bicarbonate) buffer, pH 7.4 at 37°C in 3.3 mM glucose, the islet batches were incubated in KRB-buffer in low glucose condition (3.3 mM glucose) to measure the basal insulin and in high glucose conditions (16.7 mM glucose) to measure the glucose-stimulated insulin release. A set of those conditions was tested in presence of *A. caudatus* (5 – 50 mg/ml) or *L. mutabilis* (5–20 mg/ml) extracts. Insulin release was evaluated during 60 min at 37°C in a shaking water bath [174, 182]. Aliquots of the incubation buffer were collected for insulin determination by RIA **(Papers II and III)** [178].

3.3.10 Islet Perifusion

To explore the effect of *A. caudatus* (20 mg/ml) and *L. mutabilis* (10 mg/ml) extracts on the kinetics of insulin release, batches of 40 or 50 islets were layered in a perifusion chamber between polystyrene beads where KRB buffer was perifused.

The basal insulin secretion was established during the first 20 min (KRB 3.3 mM glucose), then buffer was replaced to KRB 3.3 mM glucose plus extracts period 0 - 14 min; then to KRB 16.7 mM glucose plus extracts period 16 - 30 min; finally to KRB 3.3 mM glucose without extracts, during 20 min [182]. The AUCs for insulin release were calculated for treatment periods in presence of extracts, subtracting the basal value at the beginning of treatment **(Papers II and III)**.

3.3.11 Cytotoxicity in isolated Pancreatic Islets

To evaluate the possible toxic effect of tested concentrations of *A. caudatus* and *L. mutabilis* extracts, that could be responsible for the presence of insulin in buffer due to islet membrane destabilization and insulin leakage, islets were incubated

in presence of extracts *A. caudatus* (5 – 50 mg/ml) or *L. mutabilis* (5–20 mg/ml) in medium RPMI supplemented with 30 mg of L-glutamine, antibiotics (100 UI/ml penicillin and 0.1 mg/ml streptomycin) and 10% of heat-inactivated fetal calf serum for 1 to 24 h at 37 °C. The cell viability was determined by the reduction of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) salt to a water-insoluble purple product formazan that was further solubilized using a solution of 0.05 M of HCl in isopropanol and OD was measured at 595 nm [183-186] **(Papers I, II and III)**.

3.3.12 Mechanisms of Insulin Secretion

The mechanism of insulin secretion was evaluated using different inhibitors of key players in the glucose-dependent insulin secretion pathway. Islets were incubated in the presence of each inhibitor together with *A. caudatus* or *L. mutabilis* extracts. Conditions where the extract effect is inhibited means that this process is likely to be involved in the extract effect on insulin secretion. The inhibitors we used were: 0.25mM of Diazoxide (Dx), an opener of ATP-dependent K+ channels, active in the presence of Mg2+ [187, 188]; Dx plus 50 mM of KCl, to depolarize the β-cells [179, 189]; 10 μM of Nifedipine (Nf), a blocker of L-type Ca^{2+} channels [182, 187, 190]; 10 μM of N-[2-bromocinnamylamino)ethyl]-5-isoquinoline sulfonamide (H-89 dihydrochloride hydrate), a cell-permeable inhibitor of cAMP-dependent PKA, that binds to the ATP-binding site of the catalytic subunit, independent of magnesium [179, 191, 192]; 1.5 μM of calphostin C (Cal-C), a perylene quinone PKC inhibitor derived from the fungus *Cladosporium cladosporoides*, that targets the PKC regulatory domain [179, 189, 192, 193]; and 100 ng/ml of Pertussis toxin (Ptx), produced by *Bordetella pertussis*, an inhibitor of exocytic G proteins by ADP ribosylation of GTP-binding [26].

Following procedure previously described for insulin release, islets were incubated in presence of *A. caudatus* (20mg/ml) or *L. mutabilis* (10 mg/ml) hydroethanolic extracts in presence or absence of each inhibitor (Dx, Nf, H89 and Cal-C) for 60 min at 37°C. For PTx, islets were incubated with PTx in RPMI medium overnight, then islets were collected for insulin release assay in presence of each extract (Papers II and III) [174].

3.3.13 Glucose Uptake Evaluation

We explored the possible effect of *A. caudatus* and *L. mutabilis* extracts on insulin sensitivity in primary adipocytes isolated from epididymal fat from GK and W rats. We used a digestion protocol with type II collagenase. Isolated adipocytes were incubated with [3-^{3}H]-glucose (1 μCi/mL) for 2 h at 37°C and D-glucose solution in presence or absence of extracts and the radioactivity of ^{3}H-glucose incorporated in the de novo synthesized lipids was measured [194] **(Papers II and III)**.

3.4 Antibacterial Studies

3.4.1 Bacterial strains

Uropathogenic *E. coli* No.12 obtained from a child with acute pyelonephritis and equipped with type 1 and P-fimbriae as well as curli and cellulose [133], and the ESBL producing *E. coli* (CCUG55971) resistant to beta-lactam antibiotics, like most cephalosporins [195] and *Escherichia coli* (ATCC 25922), a control strain for antimicrobial susceptibility [196], were used to evaluate the effect of herbal extracts during infection **(Paper IV)**.

The impact of extracts on biofilm formation was studied. To promote expression of fimbriae, strains were cultured in Luria Bertani broth and agar plates without salt, for at least 24 h at 37°C prior to infection **(Paper V)** [133].

In addition, the effects of the herbal extracts were evaluated using other uropathogenic Gram-negative *Klebsiella pneumoniae* (ATCC 13883), _multidrug-resistant (MDR) *Klebsiella pneumoniae* (CCUG 58547), *Proteus mirabilis* (ATCC 29245), *Pseudomonas aeruginosa* (ATCC 27853); and Gram-positive *Enterococcus faecalis* (ATCC 29212), *Staphylococcus saprophyticus* (ATCC 15305), and *Streptococcus agalactiae* (ATCC 13813) [60] **(Papers IV and V)**.

3.4.2 Cell lines

Human bladder epithelial cell lines, T24 (HTB-4; ATCC) isolated from transitional cell carcinoma and 5637 (HTB-9; ATCC) isolated from grade II carcinoma were used as host cells for *in vitro* infection. T24 cells were cultured in McCoy's 5A medium while 5637 cells were cultured in RPMI 1640 with L-glutamine medium, both supplemented with 10% of fetal bovine serum (FBS) and were maintained at 37°C and 5% CO2 [133, 134] **(Papers IV and V)**.

3.4.3 Cell Viability Assay

Cell viability assay was determined by the colorimetric XTT reduction assay. This assay is based on the reduction of the tetrazolium salt XTT (2,3-bis (2-methoxy-4-nitro-5-sulphophenyl)-5-carboxanilide-2H-tetrazolium, monosodium salt) to a water-soluble product formazan (orange-colored), produced by the mitochondrial dehydrogenase in metabolically active cells. The direct relationship between the intensity of the formazan and the number of viable cells is measured [134, 185]. T24 cells were cultured in McCoy's culture medium in presence of *A. caudatus* (2.5 – 20 mg/ml) during 24 h **(Paper IV)**; while T24 and 5637 cells, cultured in McCoy's5A or RPMI 1640 medium, were treated with *L. mutabilis* (0.3 – 20 mg/ml) during 24 h and with *L. mutabilis* (1 mg/ml) during 48 and 72 h **(Paper V)**. Viability was calculated referred to control cells cultured in absence of extracts.

3.4.4 Antibacterial effect

The direct antibacterial effect of *A. caudatus* and *L. mutabilis* was tested using the minimum inhibitory concentration (MIC) that is defined as the lowest concentration of an antimicrobial agent that inhibits the visible growth of a microorganism after overnight incubation [133, 197]. Extracts were serially diluted in Mueller Hinton broth (MHB), tested concentration for *A. caudatus* were 250 to 1.95 mg/ml and for *L. mutabilis* 50 to 0.2 mg/ml; the final inoculum of bacteria was 5×10^5CFU/ml. Bacteria cultured only with MHB was the negative control [133] **(Papers IV and V)**.

3.4.5 Cell infection assay

T24 cells were seeded in a density of $2x10^5$ cell/well in a 24 well plate and were incubated overnight. For treatment with *A. caudatus* (10 mg/ml) or *L. mutabilis* (1 mg/ml) hydroethanolic extracts, during 24 h before infection, extracts were dissolved in the respective medium in normoglycemic glucose conditions (5mM) **(Paper V)** or hyperglycemic (11 mM) conditions **(Paper IV and V)**. Cells were infected with 10^6 CFU/ml of *E. coli* No. 12 or *E. coli* ESBL-producing strain for 30 minutes in 37°C and 5% CO2. Control cells were cultured in presence of medium only [133, 134, 198].

3.4.6 Adhesion and invasion assay

The effect of *A. caudatus* and *L. mutabilis* on the initial steps of *E. coli* infection was investigated. In adhesion assay, T24 cells, pretreated during 24 h with *A. caudatus* (10 mg/ml) or *L. mutabilis* (1 mg/ml) and infected as described before were washed with PBS to remove the non-adherent bacteria. Remaining adherent bacteria were detected after cells were lysed (0.1% triton-X-100 in PBS) and bacteria present in the lysate were cultured on blood agar and were quantified after overnight culture of serial dilution [133, 134] **(Papers IV and V)**.

Invasion assay measured the number of bacteria able to invade uroepithelial cells. Bacteria were allowed to adhere to the T24 cells, pretreated with *A. caudatus* (10 mg/ml) or *L. mutabilis* (1 mg/ml) as in the adhesion assay, and the non- adherent bacteria were washed away with PBS and replaced with fresh medium for 1 h. After allowing invasion, cells were treated with gentamycin for 30 min to kill remaining extracellular bacteria, while intracellular bacteria were not affected. Finally, the number of invading bacteria was calculated lysing the cells (0.1% triton-X-100 in PBS), and the number of intracellular bacteria was referred to the number of adhered bacteria [133, 134].

Additionally, the effect on adhesion and invasion of other bacteria strains, responsible for the development of UTI were evaluated. Extracts were dissolved in supplemented medium with normoglycemic glucose conditions (5mM) **(Paper V)** and hyperglycemic (11 mM) conditions **(Papers IV and V)**, both extracts were present throughout the entire experiment.

3.4.7 Gene expression of adherence and invasion molecules

To explore the modulation of adhesion molecules that allow bacteria adherence and invasion to host uroepithelial cells, we evaluated the gene expression of UPIa in T24 treated cells for 24 h; and gene expression of caveolin-1 and β1-integrin evaluated in T24 treated cells for 24 h followed by infection with *E. coli* No. 12 or *E. coli* ESBL-producing strains during 30 min. *A. caudatus* (10 mg/ml) treatment was done in 11 mM glucose **(Paper IV)** while *L. mutabilis* (1 mg/ml) treatment was done in 5 and 11 mM glucose **(Paper V)**. Non-treated cell controls were cultured in equivalent glucose concentrations.

The total RNA of treated and non-treated cells was extracted using a silica membrane spin column-based RNeasy mini kit, according to the manufacturer's protocol. The RNA purity and concentration were determined with a Nanodrop. 1 μg of RNA was transcribed to cDNA using the High –Capacity cDNA Reverse Transcription Kit. The relative expression was determined by quantitative real-time polymerase chain reaction (qRT-PCR) of target genes and expressed as $2^{-\Delta CT}$ to housekeeping gene and fold change as $2^{-\Delta\Delta CT}$ comparing uninfected to non-treated control [133, 134, 198]. The following probes were used (Table 2):

Table 2. Probes used to evaluate gene expression of adhesion and invasion molecules in uroepithelial cells

Gene	Probe
UPK1A (uroplakin 1a)	Hs00199638_m1, Applied Biosystems [133]
ITGB1 (β-integrin)	Hs00559595_m1, Applied Biosystems [133, 134]
CAV1 (Caveolin 1)	Hs00184697_m1, Applied Biosystems [134, 199, 200]
GAPDH	4326317E [133]
18s	Hs03003631 g1 [201]

3.4.8 Gene expression of antimicrobial peptides

Antimicrobial peptides are molecules that prevent infections by disruption of bacteria membrane, killing extracellular and intracellular bacteria. Thus, as uroepithelial cells produce antimicrobial peptide as a mechanism of innate immune response,

we explored the induction of antimicrobial peptides by treatment of uroepithelial cells culture with *A. caudatus* or *L. mutabilis*. The gene expression of antimicrobial peptides, cathelicidin LL-37, psoriasin, human β-defensin 2 and RNase7, were measured in 5637 cells treated with *A. caudatus* (10 mg/ml; 11mM glucose, hyperglycemic) **(Paper IV)** or *L. mutabilis* (1 mg/ml; 5mM, normo-glycemic and 11 mM, hyperglycemic) **(Paper V)** after 24 h of treatment by qRT-PCR, following the procedure described before. The following probes and primers were used [134, 198] (Table 3):

Table 3. Probes and primers used to evaluate gene expression of antimicrobial peptides in uroepithelial cells

Gene	Probe/Primers
CAMP (cathelicidin LL-37)	Hs00189038 m1 [133, 198]
S100A7 (psoriasin)	Hs00161488 m1 [133, 198]
DEFB4A (human β-defensin 2)	F (5' - ccctttctgaatccgc) R (5' - gagggtttgtatctcct) [202]
RNASE7 (RNase 7)	F (5'- ggagtcacagcacgaagacca) R (5' - catggctgagttg- catgcttga) [203]
ACTB (β-actin)	F (5' -aagagaggcatcctcaccct) R (5' -tacatcgctggggtgttg) [204]

3.4.9 Protein expression by immunofluorescence staining

To correlate gene expression with protein synthesis of antimicrobial peptides, protein expression was determined by immunofluorescence staining. This method provides information about the expression and location of a specific protein in the cell. It uses specific primary antibodies that target the specific molecule, then a fluorophore-conjugated secondary antibody is used to visualize the initial detection [205]. Briefly, 5637 cells grown on glass coverslips were treated *L. mutabilis* (1 mg/ml) for 24 h in both normoglycemic (5 mM) and hyperglycemic (11mM) conditions. After treatment, cells were fixed with 4% paraformaldehyde during 30 min at room temperature; to allow antibody entrance, cell membrane was permeabilized with 0.1% triton- X-100 in PBS, and blocking was done using 5% BSA in PBS for 30 min at room temperature [198]. For immune staining, cells were incubated with primary antibodies against RNase7 (1:200) or uroplakin1a (1:200) diluted in 1:1 ratio of 1 X PBS with 0.1% Tween 20 (PBS-T) and 5% BSA in PBS, overnight at 4°C. After incubation, cells were washed with 1 X PBS-T and incubated with secondary detection antibody Alexa Fluor–conjugated antibody (1:500) during 1 h at room temperature. Slides were mounted in Fluoromount G and were analyzed with a Leica SP5 confocal microscope using 63x oil immersion objective [198] **(Paper V)**.

3.4.10 Biofilm formation assay

To investigate the effect of *L. mutabilis* extract on bacterial biofilm formation, the crystal violet microtiter plate assay was used. This assay is an indirect method for bacterial adhesion and thickness of biofilm, and the method to measure is based on the matrix staining including living and dead cells [206]. *E. coli* No. 12 was grown at 37°C with 5% CO_2 for 72h in LB broth without salt, supplemented with either 5 or 11 mM glucose, or as controls with no additional glucose, and treated with *L. mutabilis* (0.4 – 1 mg/ml). At indicated time point, the planktonic cells were removed by washing with phosphate-buffered saline and bacteria that formed biofilm were stained with crystal violet (3%) [133].

After removing crystal violet and washing, a solution of acetone and ethanol was used to dissolve the remaining crystal violet and the OD was measured using spectrophotometry at 570 nm **(Paper V)**.

3.4.11 Statistical Analysis

Graph Pad Prism Software, version 6.0 was used. For antidiabetic studies, significance was analyzed by two-way analysis of variance (ANOVA) and paired Student's t-test. Correction of multiple testing was done using Bonferroni's Post Hoc Test. **(Papers I, II and III)** [174, 182]. For antibacterial studies, Student's unpaired t-test, Mann Whitney U test, and one-way ANOVA, were used [133, 134, 198] **(Papers IV and V)**.

4 RESULTS AND DISCUSSION

4.1 Antidiabetic Studies (Papers I, Paper I and II)

This part of the thesis project aims to evaluate the antidiabetic effect. First, the results of the glycemia-reducing screening in Swiss mice, are part of the **Paper I**. Further, the active plant extracts, *A. caudatus* and *L. mutabilis*, were evaluated in the T2DM animal model GK rats and its respective healthy control, W rats; results of *A. caudatus* evaluations are included in **Paper II**, while results of *L. mutabilis* evaluations are included in **Paper III**.

4.1.1 Paper I. Glycemia-reducing effects of Bolivian nutraceutical plants

Among the three kinds of extracts, both in the screening and OGTT the hydroethanolic extracts (EtOH70) of *A. caudatus*, *C. quinoa*, *L. mutabilis* and *S. sonchifolius* showed major and significant glucose-reducing effect.

Extract preparation is an important factor that should be considered to assess the plant effects [207, 208], thus we found that 70% of ethanol in distilled water solution enriched the extract with organic compounds that could be responsible for the observed effect. The phytochemical qualitative characterization of EtOH70 extracts detected high contents of anthocyanidins in *A. caudatus*, followed by phenolic compounds found also in *C. quinoa*; flavonoids and coumarins were found in *C. pallidicaule* and high contents of alkaloids in *L. mutabilis*; coumarins and anthocyanidins were found in *S. sonchifolius*, constituents reported in other studies with same species or related species which belong from same family [167].

The exploration of the effect of EtOH70 extracts on *in vitro* insulin secretion, performed in batches of mice pancreatic islets showed that extracts have different effects on glucose homeostasis since only extracts of *A. caudatus*, *L. mutabilis* and *C. quinoa* increased insulin release (Figure 8).

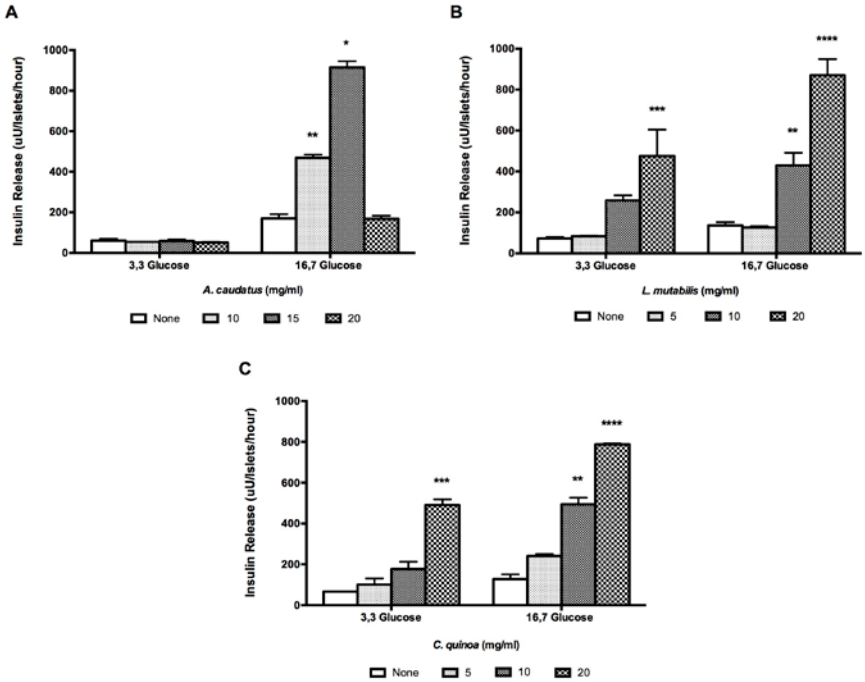

Figure 8. A. caudatus, L. mutabilis and C. quinoa EtOH70 extracts stimulates insulin release. *Swiss mice islets were incubated in KRB (3.3 mM or 16.7 mM glucose) plus EtOH70 extracts of A. caudatus (A), L. mutabilis (B) and C. quinoa (C). Data are presented as means ± SEM, of duplicates from three independent experiments. *p< 0.05, ** p<0.01, *** p<0.001, **** p<0.0001, compared to negative control.*

A. caudatus seeds water decoction [209] and methanolic extract [210] have been reported to inhibit α-amylase. However, in our study *A. caudatus* stimulated insulin release in high glucose conditions (16.7 mM), but not in low glucose conditions, that could make *A. caudatus* ideal for potential therapeutic use.

L. mutabilis reduces glycemia in patients with intolerance to glucose [211] and in patients with type 2 diabetes, due to the alkaloid content [212]. *C. quinoa* inhibits the α-glucosidase with no effect on pancreatic α–amylase [213] and in high fructose-treated Wistar rats, reduced the lipid profile and glycemia [214]. In our hands, *L. mutabilis* and *C. quinoa* stimulated insulin release in both low and high glucose conditions, and their effect was concentration-dependent. Thus, their effects were similar to those of sulphonylurea drugs [89]. None of the hydroethanolic extracts showed acute *in vivo* toxicity and *in vitro* experiments used concentrations lower than the media cytotoxic concentration.

In summary, we provide information about the glycemia-reducing properties of plants under study in non-fasted and in mice with induced-hyperglycemia; this effect is partially mediated by the stimulation of insulin secretion in islets treated with *A. caudatus*, *L. mutabilis* and *C. quinoa*. Results of screening studies confirmed literature reports and oriented further experiments to explore the effect on insulin secretion in a T2DM model.

4.1.2 Paper II. Amaranthus caudatus stimulates insulin secretion in Goto-Kakizaki rats, a model of Diabetes Mellitus type 2

In vivo evaluations showed that a single oral administration of *A. caudatus* (2000 mg/kg b.w.) hydroethanolic extract augmented serum insulin during the first 30 min of the OGTT in GK rats 1.46 fold and in W rats1.64 fold and the glucose tolerance improved with a reduction of the AUC of the glucose curve of 24% in GK rats and 40% in W rats.

In GK rats, the oral treatment with *A. caudatus* (1000 mg/kg b.w.) during 21 days reduced the non-fasting glycemia and improved the glucose tolerance with a reduction of the AUC of 24% in GK rats, and by increasing serum insulin levels 2.3-fold at day 20. In W rats the AUC was reduced 45% and serum insulin increased 2.2-fold, with no signal of hypoglycemia. Besides, *A. caudatus* reduced the percentage of HbA1c, 19.8 % in GK rats (Figure 9A) and 10.7% in W rats (Figure 9C), evidence of the *A. caudatus* contribution to long-term glycemic control. The augmented serum insulin suggests that *A. caudatus* affects insulin release, as proved by the improvement of glucose-dependent insulin secretion in pancreatic islets isolated from treated GK (Figure 9B) and W rats (Figure 9D).

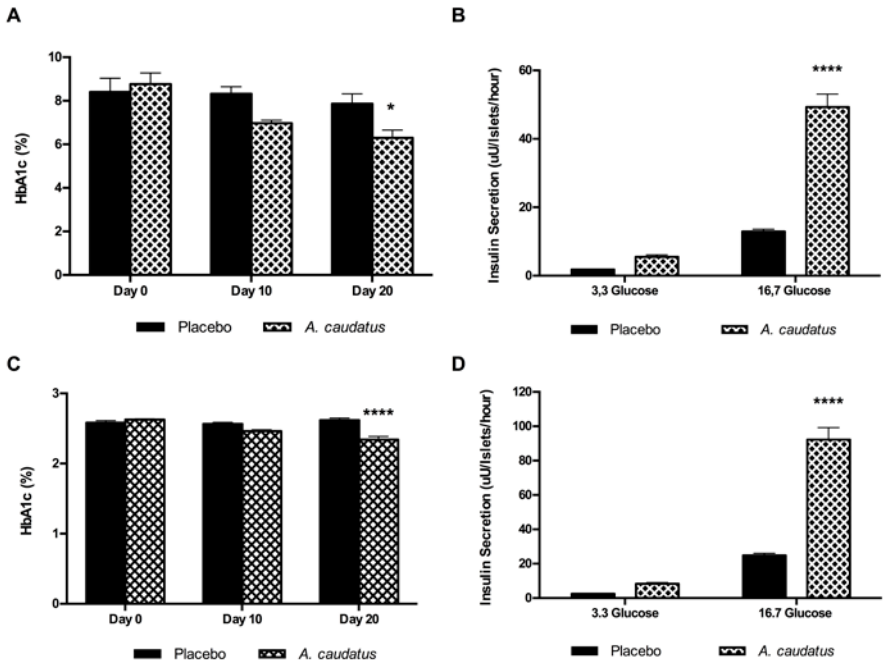

Figure 9. A. caudatus oral treatment during 21 days reduces the plasma HbA1c and improved insulin secretion in pancreatic islets. *HbA1c measured in time cero, 10 and 20 days in GK rats (***A***) and in W rats (***C***) (n = 6). Pancreatic islets isolated at the endpoint from GK rats (***B***) and W rats (***D***) were cultured in low and high glucose (n = 4). Data are presented as means ± SEM and significances * p < 0.05, **** p < 0.0001 compared to placebo group; ## p < 0.01, compared to values from the same group.*

Previous reports found that a methanolic extract of *A. caudatus* entire plant and methanolic extract of seeds inhibit the α-amylase activity [210, 215]. In SZT-diabetic rats, the methanol extract of *A. caudatus* leaves decreases glycemia [216]. A similar effect on glycemia reduction is attributed to other species from *Amaranthus* genus [217-221]. Our results support the traditional use and the scientific evidence available about the effect on glycemia reduction and the other parameters measured explain the effect by an improvement on β-cell insulin release, i.e. a primary metabolic defect in GK rats [9]. According to the phytochemical analysis, the *A. caudatus* extract has constituents in concordance to reports of *A. caudatus* or other species from the Amaranthus family [216, 222, 223]. Besides, sub-acute toxicity studies did not show a toxic effect in tested conditions.

In vitro studies showed that *A. caudatus* stimulated the insulin secretion in batch-incubated islets; with a concentration-dependent effect observed in both GK and W rat in high glucose conditions. In low glucose conditions, a significant but lower

effect was observed (Figure 10A-C). Furthermore, *A. caudatus* enhanced the basal insulin secretion in perifused islets in low glucose and was further stimulated in high glucose conditions, but when the AUC was calculated, significant differences were only observed in W rats. Interestingly, *A. caudatus* effect was reversible since insulin secretion returned gradually to the basal level when the extract was removed from the perifusion buffer (Figure 10B-D). These results are evidence that *A. caudatus* does not cause any toxic effect on β-cells followed by insulin leakage; a similar effect has been described for isolated compounds from other plant extracts [174, 182, 224].

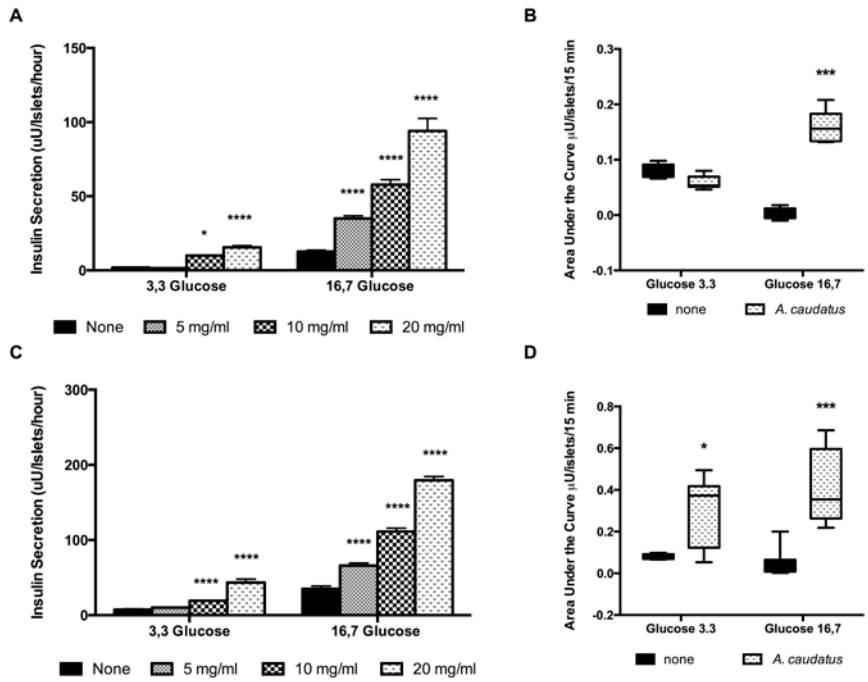

Figure 10. A. caudatus stimulates the in vitro insulin secretion in batch and perifused pancreatic islets. *Insulin secretion in presence of A. caudatus (5–20 mg/ml) was evaluated in GK rat islets (***A***) and W rat islets (***C***) (n = 8). The AUC of the insulin secretion in perifused islets with A. caudatus (20 mg/ml) form GK (***B***) and Wistar (***D***) rats from time 0 to 14 min (3.3 mM glucose) and time 16 to 30 min (16.7 mM glucose) (n=4). Data are presented as means ± SEM and significances *p<0.05,**p<0.01,***p<0.001, **** p < 0.0001 compared to untreated islets.*

The exploration of the mechanism of action showed that *A. caudatus* (20 mg/ml) effect is glucose-independent that mainly involves PKA and PKC systems, and partially the L-type Ca^{2+} channels opening and G-coupled exocytosis (Table 4).

Table 4. Effect of inhibitors of insulin secretion pathway, Nifedipine (Nf), H-89 dihydrochloride hydrate (H89), Claphostine C (Cal-C) and Pertussis Toxin (Ptx) in pancreatic islets from GK and W rats.

	GK rats		W rats	
Glucose	**3.3 mM**	**16.7 mM**	**3.3 mM**	**16.7 mM**
Conditions				
None	2.60 ±0.37	12.18± 0.66	6.88±0.79	35.05±2.79
A. caudatus (20 mg/ml)	15.50 ±0.85	92.75±5.82	38.77±3.79	164.48±4.65
Nf (10 μM)	2.82± 0.34	7.97±0.75	4.17±0.24	14.47±1.28
A. caudatus (20 mg/ml) + Nf (10 μM)	10.55±1.01	57.48±3.36****	21.71±2.17**	95.57±8.72****
H89 (10 μM)	4.33±0.53	6.69±0.49	10.97±1.17	19.63±1.72
A. caudatus (20 mg/ml) + H89 (10 μM)	6.94±0.58****	17.85±1.59****	9.79±1.58****	19.10±1.30****
Cal-C (1.5 μM)	3.14±0.33	4.23±0.52	7.07±0.54	15.74±1.69
A. caudatus (20 mg/ml) + Cal-C (1.5 μM)	4.48±0.34****	16.45±0.74****	13.04±0.60****	23.53±2.48****
Ptx (100 ng/mL)	2.48±0.30	3.50±0.26	2.47±0.31	3.50±0.26
A. caudatus (20 mg/ml) + Ptx (100 ng/ml)	6.68±0.33**	67.22±3.13***	29.94±1.06***	146.42±6.87***

Insulin concentration (μU/islet) are presented as means ± SE (n = 8) of A. *caudatus plus each inhibitor.*
****p< 0.05, ** p<0.01, *** p<0.001, **** p<0.0001 compared to A.*** *caudatus alone.*

The activation of PKA and PKC promotes the increase of highly sensitive to Ca^{2+} insulin vesicles [192, 225], a mechanism that might explain the increase of insulin release in presence of *A. caudatus*. Additionally, the intracellular Ca^{2+} increase and the exocytosis mediated by G-proteins [226] are partially required for *A. caudatus* effect during the final stages of insulin release.

The *A. caudatus* inhibition mediated by Ptx might be related to the inhibition of other G-proteins, that is finally required for kinases activation; Gs protein that mediates PKA activation by the activation of adenylate cyclase and Gq that mediates PLC

activation, i.e. events that finally activates PKC [225, 226]; thus, the exploration of the role of those G-proteins in *A. caudatus* effect has to be further evaluated.

The membrane depolarization of β-cells was not a primary mechanism attributed to *A. caudatus* since a high concentration of KCl plus diazoxide did not inhibit the *A. caudatus* effect. Moreover, no effect was observed on glucose uptake in primary adipocytes.

4.1.3 Paper III. Lupinus mutabilis extract exerts anti-diabetic effect by improving insulin release in type 2 diabetic Goto-Kakizaki rats

A single oral administration of *L. mutabilis* hydroethanolic extract (2000 mg/kg b.w.) improved glucose tolerance, with a reduction of 25% of the AUC of glucose under the OGTT and augmented serum insulin 2.08-fold, in GK rat; a similar effect was observed in W rats. The effect was dose-dependent and the increment in serum insulin started already after the extract administration, meaning that *L. mutabilis* effect is glucose-independent. Interestingly, the effect did not induce hypoglycemia, since glycemia at the end of the test were not lower than fasted values.

The long-term treatment with *L. mutabilis* (1000 mg/kg b.w.) improved glucose metabolism, evidenced by a reduction of the AUC of glucose during the OGTT in both types of rats, and a significant reduction of 12% of the non-fasting glucose observed only in GK rats, meaning that *L. mutabilis* controlled high glycemia levels present in GK rats [9], a condition not present in W rats. Additionally, the HbA1c percentage was reduced, and serum insulin was increased (Figure 11A-C). Moreover, *L. mutabilis* improved the insulin release in pancreatic islets from treated animals in low and high glucose conditions, suggesting that the glycemia control is secondary to the promotion of β-cell function (Figure 11B-D).

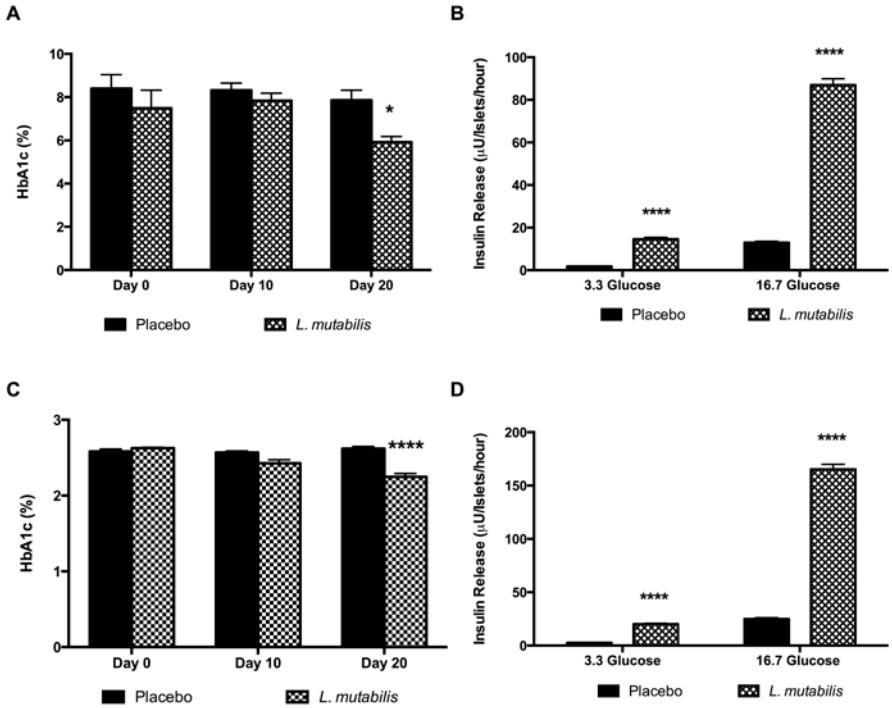

Figure 11. L. mutabilis oral treatment during 21 days reduces the plasma HbA1c and improved insulin secretion in pancreatic islets. *HbA1c measured in time cero, 10 and 20 days in GK rats (***A***) and W rats (***C***) (n = 6). Pancreatic islets isolated at the endpoint from GK rats (***B***) and W rats (***D***) were cultured in low and high glucose (n = 4). Data are presented as means ± SEM and significances * p < 0.05, **** p < 0.0001 compared to placebo group; ## p < 0.01, compared to values from the same group.*

These findings support the use of daily consumption of components present in *L. mutabilis* extract to control hyperglycemia in diabetic patients [211, 212]. Traditionally, the water in which *L. mutabilis* seeds are washed controls glycemia after meals [227]. Our results support the *L. mutabilis* acute mechanism that augments serum insulin in a glucose-independent way, and its constituents might have a rapid absorption to target the pancreas to stimulate insulin release.

In vitro experiments showed that *L. mutabilis* has a glucose-independent effect on insulin release in GK rats, while W rats islets were more sensitive (Figure 12 A-C). Interestingly, the highest *L. mutabilis* concentration used showed less effect; possible due to the interaction of inhibitory compounds or to the possible toxicity of the alkaloid content, present in the extract [228]. However, the *in vitro* cytotoxicity studies showed that tested concentrations were not toxic in the time period used in our experiments. *L. mutabilis* improved insulin release in perifused GK islets, the

effect started gradually in low glucose and was enhanced in high glucose; when the extract was removed, insulin secretion went back to basal levels, evidence that *L. mutabilis* does not alter β-cell structure or function (Figure 12B-D).

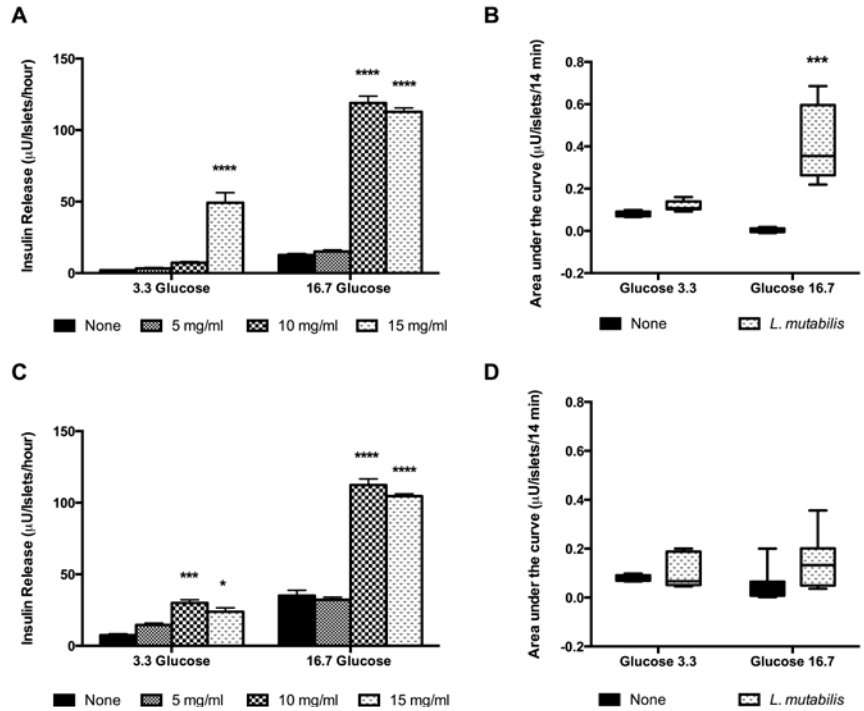

Figure 12. L. mutabillis stimulates the in vitro insulin secretion in batch and perifused pancreatic islets. *Insulin secretion in presence of L. mutabillis (5–15 mg/ml) was evaluated in GK rat islets (***A***) and W rat islets (***C***) (n = 8). The AUC of the insulin secretion in perifused islets from GK (***B***) and Wistar (***D***) rats from time 0 to 14 min (3.3 mM glucose) and time 16 to 30 min (16.7 mM glucose) (n=4). Data are presented as means ± SEM and significances *p<0.05,**p<0.01,***p<0.001, **** p < 0.0001 compared to untreated islets.*

In vitro evaluation of the mechanism of action using blocking components of the insulin secretion pathway showed that *L. mutabilis* effect modulates initial steps of insulin secretion pathway i.e. the closure of K-ATP channels and the opening of L-type Ca^{2+} channels. Several reports describe the effects on stimulation of insulin release of different Lupinus species, mainly due to their alkaloid content i. e. sparteine, lupanine and synthetic derivatives, an effect that is inhibited by diazoxide [229-232]; while *L. mutabilis* modulation of voltage-dependent Ca^{2+} channels that might trigger exocytosis of insulin-containing granules, has not been reported before (Table 5).

Table 5. Effect of inhibitor of insulin secretion pathway, Diazoxide (Dx) and Nifedipine (Nf) in pancreatic islets from GK and W rats.

	GK rats		W rats	
Glucose	**3.3 mM**	**16.7 mM**	**3.3 mM**	**16.7 mM**
Conditions				
none	3.96±0.46	12.70±0.72	7.30±1.24	38.51±2.97
L. mutabilis (10 mg/ml)	7.21±0.46	104.71±6.96	27.78±1.80	103.33±4.94
Dx (0.25 mM)	2.93±0.48	10.58±1.05	3.74±0.19	18.68±0.69
Dx (0.25 mM) + KCl (50 mM)	6.70±0.60	40.22±1.78	9.27±1.07	81.82±2.33
L. mutabilis (10 mg/ml) + Dx (0.25 mM)	17.17±1.91	60.36±3.25****	20.49±1.20****	53.08±4.33****
L. mutabilis (10 mg/ml) + Dx (0.25 mM) + KCl (50 mM)	12.03±0.54	47.68±2.13****	34.07±2.62****	61.62±3.43****
Nf (10 μM)	4.76±0.57	8.44±0.65	4.11± 0.29	17.42±1.10
L. mutabilis (10 mg/ml) + Nf (10 μM)	18.31±1.33	31.17±3.14****	18.21±1.89	39.77±1.53****

Insulin concentration (μU/islet) are presented as means ± SE (n = 8) of A. *caudatus plus each inhibitor.*
****p< 0.05, ** p<0.01, *** p<0.001, **** p<0.0001 compared to A.*** *caudatus alone.*

The results of the phytochemical analysis are in concordance with the literature, the hydroethanolic crude extract main components are alkaloids [212, 229, 233, 234]; therefore, the observed effect might be attributed to the presence of alkaloids. Since alkaloids found in Lupinus species are reported as neurotoxins [235], toxicity studies were conducted. *In vivo* sub-acute toxicity showed that tested dose (1000 mg/kg b.w.) did not alter hematological, biochemical or behavior parameters, and *in vitro* cytotoxicity evaluations showed that used concentrations were not toxic.

Furthermore, *L. mutabilis* effect in GK rat islets involves the activation of both the PKA and PKC, and G-protein mediated exocytosis, only in high glucose. In W rat islets, those systems are involved in both low and high glucose conditions. PKA and PKC activation promote the increase of highly sensitive to Ca^{2+} insulin vesicles [192, 225] and G proteins are involved in the last steps of insulin release by membrane fusion mediated by SNARE proteins; thus both events are strongly modulated by *L. mutabilis* (Table 6).

Table 6. Effect of inhibitor of insulin secretion pathway, H-89 dihydrochloride hydrate (H89), Calphostine C (Cal-C) and Pertussis Toxin (Ptx) in pancreatic islets from GK and W rats.

	GK rats		**W rats**	
Glucose	**3.3 mM**	**16.7 mM**	**3.3 mM**	**16.7 mM**
Conditions				
None	3.96±0.46	12.70±0.72	7.30±1.24	38.51±2.97
L. mutabilis (10 mg/ml)	7.21±0.46	104.71±6.96	27.78±1.80	103.33±4.94
H89 (10 µM)	4.33±0.53	6.69±0.49	10.96±1.17	19.63±1.72
L. mutabilis (10 mg/ml) + H89 (10 µM)	4.44±0.40	13.86±1.0****	11.33±0.72****	35.31±1.51****
Cal-C (1.5 µM)	3.14±0.33	4.23±0.51	7.07±0.54	15.74±1.70
L. mutabilis (10 mg/ml) + Cal-C (1.5 µM)	12.86±0.57	30.57±1.54****	25.64±1.06*	63.07±2.65****
Ptx (100 ng/mL)	2.47±0.30	3.50±0.26	2.52±0.32	3.67±0.34
L. mutabilis (10 mg/ml) + Ptx (100 ng/ml)	3.11±0.30	7.37±0.92****	16.74±0.94*	38.42±2.67****

Insulin concentration (µU/islet) are presented as means ± SE (n = 8) of A. *caudatus plus each inhibitor.*
****p< 0.05, ** p<0.01, *** p<0.001, **** p<0.0001 compared to A.*** *caudatus alone.*

4.1.4 Graphical summary of Antidiabetic effect

A. caudatus and *L. mutabilis* hydroethanolic extracts effects on insulin secretion modulate certain points of the insulin secretion pathway with punctual differences on critical events, a summary of the proposed mechanism for both extracts is shown in figure 13.

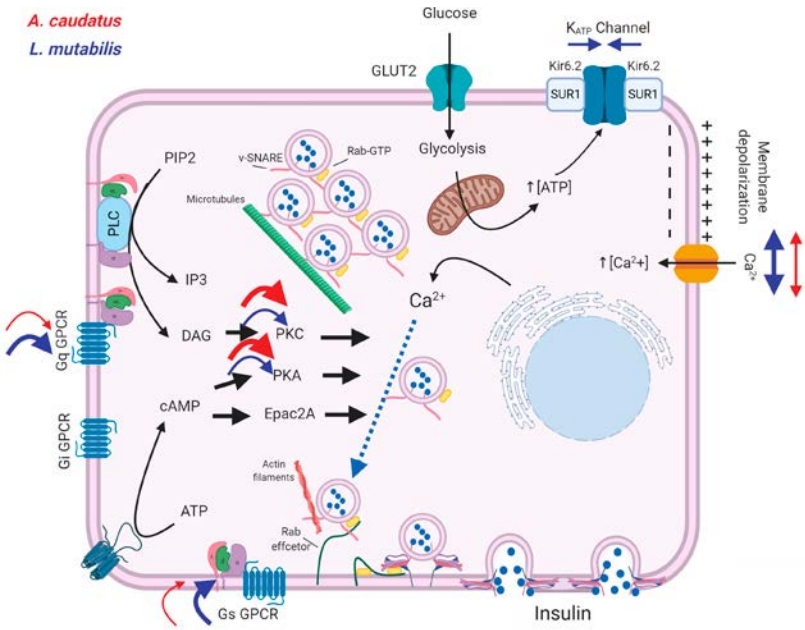

Figure 13. Mechanisms of A. caudatus and L. mutabilis on insulin secretion. *Critical points along the insulin secretion pathway modulated by A. caudatus (red arrows) and L. mutabilis (blue arrows). Intensity of arrows is related with the relevance of each specific point.*

4.2 Antibacterial Activity Studies (Papers IV and V)

UTIs are frequent infections in diabetic patients and maybe promoted as a complication of glucose imbalance and by a defect in the immune response [236]. The globally increasing antibiotic resistance is a threat to successful treatment of the patient. Therefore, the search for new strategies to prevent or treat those infections is needed. In this part of the study, we explored the same hydroethanolic extracts of *A. caudatus* **(Paper IV)** and *L. mutabilis* **(Paper V)** as above, for the possible impact on UTI.

4.2.1 Paper IV. Amaranthus caudatus extract inhibits the invasion of E. coli into uroepithelial cells

A. caudatus hydroethanolic extract did not show any direct antibacterial effect. The results are contrary to studies using another Amaranth species i.e. *Amaranth hybridus* [237] and *Amaranthus retraflexus* [238] which have a direct antibacterial effect against ESBL-producing *E. coli*. isolates.

The exploration of the relationship between bacteria and host during infection is another approach to test further activities of medicinal plants when the direct antibacterial effect has not been reported by medicinal plants [239, 240]. The impact of *A. caudatus* on the host-bacteria interaction was investigated in uroepithelial cells pre-treated with *A. caudatus* for 24 hours. We sought to study the possible protection against the initial stages of the infection process, adhesion and invasion. The tested concentrations did not exert any cytotoxic effects. *A. caudatus* treatment (10 mg/ml), significantly reduced bacterial adhesion of *E. coli* #12 and the ESBL producing *E. coli* while no effect was observed on invasion. A similar effect on bacterial adhesion was reported for other medicinal plants, *Citrus reticulata* reduces bacterial invasion [134]. *Clinopodium bolivianum* inhibits bacterial adhesion, which was attributed to the flavonoids content [133]. The phytochemical analysis of *A. caudatus* revealed minor amounts of polyphenols as a part of its constituents, among them flavonoids are chemicals that have been related to decreased adherence of UPEC to uroepithelial cells [241, 242].

Uroepithelial umbrella cells express surface glycoprotein UPIa that mediates bacterial adhesion by interaction with the subunit FimH of the type 1 fimbriae [58, 243], its reduction may, therefore, interfere with UPEC binding [244, 245]. The treatment of non-infected T24 cells with *A. caudatus* decreases UPIa gene expression after 24 h, this reduction is still observed during the infection process in the presence of *A. caudatus,* resulting in decreased *E. coli* adhesion. It has been reported that upon infection, the interaction between bacteria and cell receptors, triggers signal transduction that increases UPIa gene expression [52, 60]. Thus, the *A. caudatus* effect might interfere with the bacterial-induced signal transduction that promotes bacterial adhesion. A similar reduction was reported for *Clinopodium bolivianum* [133].

During the bacterial invasion process, β1-integrins and caveolin-1 proteins interact with FimH to promote UPEC invasion of bladder epithelial cells [57, 58]. In our study, *A. caudatus* pretreated T24 cells reduced gene expression of caveolin-1, both in non-infected and infected cells, while β1 integrin expression was not affected. Caveolin-1 proteins are located in the lipid rafts, a cell membrane domain which is responsible for the transduction of external signals [57]. The lipid rafts have also been reported to influence bacterial invasion through endocytosis [54]. Therefore, a reduced expression of caveolin-1 mediated by *A. caudatus* might disrupt bacterial infection. The invasion process allows the establishment of IBCs, which promotes UPEC persistence [52], although bacteria, often phenotypically changed, can be released and infect nearby cells. It is, therefore, possible that *A. caudatus* might reduce the risk of recurrence of UTI [58, 246]. Finally, we further explored the expression of antimicrobial peptides, but we did not observe any effect of the tested antimicrobial peptides.

The strategy to prevent infection is an approach that is currently being explored, using vaccines to block FimH [46, 247]. Mannosides that mimic the natural receptor for type 1 pili specifically bind UPEC [248] and medicinal herbs may act on the host cells having similar result [48]. Therefore, results of *A. caudatus* treatment contribute to the promising strategies to prevent infection UPEC infection. Further studies are required to explore *A. caudatus* effect using *in vivo* UTI infection model.

4.2.2 Paper V. Lupinus mutabilis Edible Beans Protect against Bacterial Infection in Uroepithelial Cells

To further broaden our search for possible alternative treatment strategies, we investigated the edible bean *L. mutabilis.* We did not observe any direct antibacterial effect, on uropathogenic bacteria when testing *L. mutabilis* hydroethanolic extract, up to 125 mg/ml. Then, we explored the indirect effect on the initial steps of bacterial infection.

To rule out the toxic effect on the uroepithelial cells, we explored the viability of T24 and 5637 cells in the presence of *L. mutabilis* extract (1 mg/ml). After 24 h of exposure, the viability of treated cells was similar to untreated cell (99%), and when the concentration increased up to 20 mg/ml, viability was still around 80%. Moreover, following prolonged exposure of *L. mutabilis* up to 72 h, did not affect the cell viability which similar as untreated cells. We, therefore, conclude that there was no effect on the cell cycle or the metabolism, measured by XTT assay. Interestingly, studies have reported toxicity of alkaloids in *L. mutabilis* [235]. In our *in vitro* model, the concentrations were, however, not high enough to induce toxic effects.

We then explored the indirect effect on the initial steps of bacterial infection to the host. When investigating the effect on bacterial infection on uroepithelial cells, T24, we treated the cells with *L. mutabilis* (1 mg/ml), during 24 h prior to infection, and demonstrate reduced UPEC adherence. To confirm this finding, we tested other uropathogenic Gram-negative bacteria, like, ESBL-producing *E. coli, K. pneumoniae,* MDR *K. pneumoniae*, *P. mirabilis* and Gram-positive bacteria like *S. saprophyticus*, in both normal and high glucose conditions adhesion of mentioned bacteria was reduced, while adhesion of *P. mirabilis* was reduced only in normal glucose; the invasion process of all bacteria strain was not inhibited. The inhibition of bacterial adhesion observed in high glucose (11mM) indicates that *L.mutabilis* may protect against UPEC in hyperglycemic conditions observed in T2DM patients. Even though *E. coli* is the main microorganism responsible for UTI, other bacteria can also cause this infection [50] thus, *L.* mutabilis prevented adhesion to uroepithelial cells not only of Gram-negative but also Gram-positive bacteria, further studies to explore the specific events during bacterial adhesion need to be performed, since bacteria have their specific mechanism of infection.

Moreover, since *L. mutabilis* prevents adhesion of ESBL and MDR strains, the extracts might provide an alternative or complementary treatment strategy, especially to antibiotic-resistant bacteria.

To explore the changes induced by *L. mutabilis* in host cells, we evaluated the expression of UPIa. In our study, *L. mutabilis* down regulated UPIa gene and protein expression in 5637 cells, treated during 24 h, prior to infection. The effect was observed both in normal and high glucose conditions. In parallel, expression of molecules important in the bacterial invasion was also evaluated, Caveolin-1 and β1-integrin, but no changes were observed. These findings support our initial results where only the adhesion process was inhibited. *L. mutabilis* effect observed in both normal and high glucose concentrations, i.e. a condition that is similar to what is observed in diabetic patients, where they present glycosuria, might represent a promising product to prevent UTI in those individuals.

Interestingly, *L. mutabilis* up-regulated the AMP RNase 7 gene expression and protein levels, both in low and high glucose conditions, while no effect was observed on LL-37 or human β-defensin 2. AMPs inhibit bacterial infection due to killing extra and intracellular bacteria by disruption of bacterial membrane [71]. RNase 7 is an AMP normally expressed in uroepithelial cells, where it has a bactericidal effect against Gram-negative and positive bacteria, including the multidrug-resistant strains [78]. Thus, we can conclude that *L. mutabilis* reduces the number of infecting bacteria due to bacterial lysis induced by RNase7. The remaining viable bacteria are prevented to adhere to the uroepithelium, because of the reduction of UPIa. This effect might be important to control UTI in the context of glycosuria present in diabetic patients. The high prevalence of UTI in diabetic patients is partly explained by the impairment of antimicrobial peptides function, as RNase 7 is released in an insulin dependent manner [78, 203]. In this context, *L. mutabilis* might offer an alternative or adjuvant strategy to prevent UTI in diabetic patients.

Moreover, *L. mutabilis* (1 mg/ml) inhibited *E. coli* No. 12 biofilm formation, with no impact on bacterial growth, although the effect was observed only in normal glucose conditions. The ability of UPEC to form biofilm, allows bacterial persistence and difficulties to successful treatment and risk of recurrent infections [249]. Similar results are attributed to herbal extracts [135] and components as to polyphenols [242], sugars and fructose that suppress mannose-sensitive fimbrial adhesins [250]. Moreover, biofilm inhibition was observed with sub-inhibitory concentrations of LL-37 [82]. Biofilm inhibition is one mechanism that contributes to prevent recurrent infection [61], pro-inflammatory cytokines are related to biofilm inhibition which promotes planktonic growth [49]. Thus, *L. mutabilis* effect on cytokine expression and the exploration of the specific mechanisms behind the biofilm formation are needed.

4.2.3 Graphical summary of the antibacterial effect

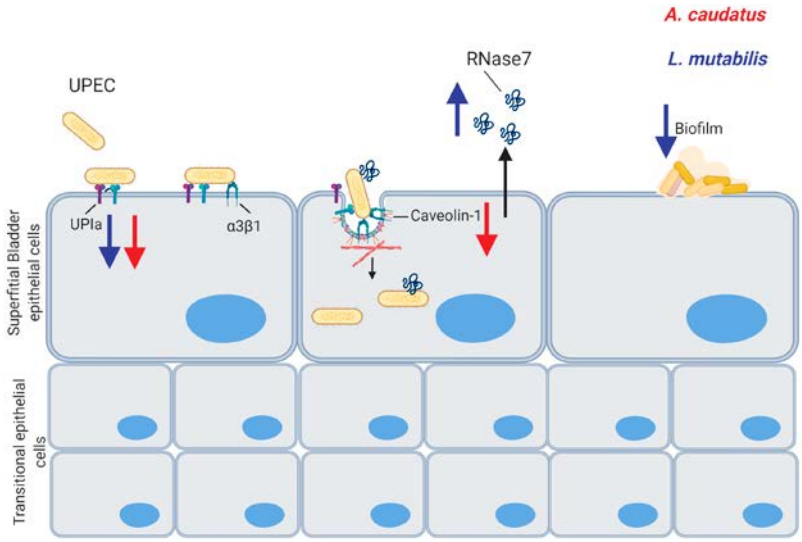

Figure 14. Mechanisms of A. caudatus and L. mutabilis antibacterial effect. *Points along the UPEC infection pathways modulated by A. caudatus are indicated in red arrows; a decrease of UPIa and caveolin-1 gene expression. Effects of L. mutabilis are indicated in blue arrows; decrease of UPIa gene and protein expression, upregulation of gene and protein expression of RNase7, and inhibition of biofilm formation.*

5 CONCLUSIONS

1. In the screening studies, the hydroethanolic extract of all plants tested showed a strong glycemia-reducing effect in normoglycemic Swiss mice. The effect involved the stimulation of insulin secretion in islets treated with *A. caudatus*, *L. mutabilis* and *C. quinoa*.
2. Based on screening studies, we further selected to continue the antidiabetic studies. *A. caudatus* and *L. mutabilis* improved glucose tolerance in the diabetic GK rat model, effects secondary to the long-term glycemia control reflected as reduction of the Hb1Ac and increase of insulin secretion. The mechanisms behind these effects differ somewhat between extracts. *A. caudatus* stimulated insulin secretion using protein kinases A and C activation, and partially involving intracellular calcium increase and G protein-coupled exocytosis. The *L. mutabilis* effect depended on L-type calcium channels, PKC and PKA systems, G protein-coupled exocytosis and was partially mediated by K-ATP channels of the β-cells. Thus, both extracts are promising candidates for the treatment of type 2 diabetes mellitus.
3. *A. caudatus* and *L. mutabilis* extracts might contribute to the prevention of common infections like UTI caused by uropathogenic *E. coli*. *A. caudatus* protects the uroepithelium from invading bacteria, including drug-resistant strains by downregulating the membrane proteins, uroplakin1a and caveolin-1, which contribute to adhesion and invasion in uroepithelial cells. *L. mutabilis* further prevents bacterial adhesion, irrespective of glucose concentrations. This effect is explained by downregulation of the adhesion molecule, uroplakin-1a and up-regulation of the antimicrobial peptide RNase7. Moreover, *L. mutabilis* inhibited bacterial biofilm. These results are important findings to understand the interaction between the host and the bacteria in the pathogenesis of UTI. It may also serve as a possible alternative preventive strategically approach.

In summary, our results provide evidence of the pharmacological activity of the plant extracts tested, supporting the nutraceutical potential of *A. caudatus* and *L. mutabilis* in the treatment of type 2 diabetes mellitus and hopefully also to prevent UTI in hyperglycemia, mimicking the diabetic condition.

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7 REFERENCES

1. Ostenson, C.G., *The pathophysiology of type 2 diabetes mellitus: an overview.* Acta Physiologica Scandinavica, 2001. **171**(3): p. 241-247.

2. Zaccardi, F., et al., *Pathophysiology of type 1 and type 2 diabetes mellitus: a 90-year perspective.* Postgraduate Medical Journal, 2016. **92**(1084): p. 63-69.

3. Kaku, K., *Pathophysiology of type 2 diabetes and its treatment policy.* Japan Medical Association Journal, 2010. **53**: p. 41-46.

4. Robertson, R.P., et al., *Glucose toxicity in beta-cells: type 2 diabetes, good radicals gone bad, and the glutathione connection.* Diabetes, 2003. **52**(3): p. 581-587.

5. Kim, J.W. and K.H. Yoon, *Glucolipotoxicity in Pancreatic beta-Cells.* Diabetes & Metabolism Journal, 2011. **35**(5): p. 444-450.

6. Rabhi, N., et al., *Role of the unfolded protein response in beta cell compensation and failure during diabetes.* Journal of Diabetes Research, 2014. **2014**: p. 1-11.

7. Pajvani, U.B. and D. Accili, *The new biology of diabetes.* Diabetologia, 2015. **58**(11): p. 2459-2468.

8. Cersosimo, E., et al., *Pathogenesis of Type 2 Diabetes Mellitus*, in *Endotext*, L.J. De Groot, et al., Editors. 2000: South Dartmouth, USA.

9. Östenson, C.G., et al., *Abnormal insulin secretion and glucose metabolism in pancreatic islets from the spontaneously diabetic GK rat.* Diabetologia, 1993. **36**(1): p. 3-8.

10. Szuszkiewicz-Garcia, M.M. and J.A. Davidson, *Cardiovascular disease in diabetes mellitus: risk factors and medical therapy.* Endocrinology & Metabolism Clinics of North America, 2014. **43**(1): p. 25-40.

11. Sacks, F.M., et al., *Association between plasma triglycerides and high-density lipoprotein cholesterol and microvascular kidney disease and retinopathy in type 2 diabetes mellitus: a global case-control study in 13 countries.* Circulation, 2014. **129**(9): p. 999-1008.

12. Zheng, Y., S.H. Ley, and F.B. Hu, *Global aetiology and epidemiology of type 2 diabetes mellitus and its complications.* Nature Reviews Endocrinology, 2018. **14**(2): p. 88-98.

13. Sluik, D., et al., *Lifestyle factors and mortality risk in individuals with diabetes mellitus: are the associations different from those in individuals without diabetes?* Diabetologia, 2014. **57**(1): p. 63-72.

14. Tajima, R., et al., *High cholesterol intake is associated with elevated risk of type 2 diabetes mellitus – A meta-analysis.* Clinical Nutrition, 2014. **33**(6): p. 946-950.

15. Hemmingsen, B., et al., *Diet, physical activity or both for prevention or delay of type 2 diabetes mellitus and its associated complications in people at increased risk of developing type 2 diabetes mellitus.* The Cochrane Database of Systematic Review, 2017. **12**: p. 1-3.

16. Siddiqui, A., et al., *Endocrine stress responses and risk of type 2 diabetes mellitus.* Stress, 2015. **18**(5): p. 498-506.

17. Eriksson, A.K., et al., *Psychological distress and risk of pre-diabetes and Type 2 diabetes in a prospective study of Swedish middle-aged men and women.* Diabetic Medicine, 2008. **25**(7): p. 834-842.

18. Eriksson, A.K., et al., *Work stress, sense of coherence, and risk of type 2 diabetes in a prospective study of middle-aged Swedish men and women.* Diabetes Care, 2013. **36**(9): p. 2683-2689.

19. Shaw, J.E., R.A. Sicree, and P.Z. Zimmet, *Global estimates of the prevalence of diabetes for 2010 and 2030.* Diabetes Research and Clinical Practice, 2010. **87**(1): p. 4-14.

20. Cho, N.H., et al., *IDF Diabetes Atlas: Global estimates of diabetes prevalence for 2017 and projections for 2045.* Diabetes Research and Clinicla Practice, 2018. **138**: p. 271-281.

21. Carrillo-Larco, R.M., et al., *The risk of mortality among people with type 2 diabetes in Latin America: A systematic review and meta-analysis of population-based cohort studies.* Diabetes Metabolism Research and reviews, 2019. **35**(4): p. 1-11.

22. Muoio, D.M. and C.B. Newgard, *Molecular and metabolic mechanisms of insulin resistance and β-cell failure in type 2 diabetes.* Nature Reviews Molecular Cell Biology, 2008. **9**(3): p. 193-205.

23. Ostenson, C.G. and S. Efendic, *Islet gene expression and function in type 2 diabetes; studies in the Goto-Kakizaki rat and humans.* Diabetes, Obesity and Metabolism, 2007. **9** (2): p. 180-186.

24. Winzell, M.S. and B. Ahren, *G-protein-coupled receptors and islet function-implications for treatment of type 2 diabetes.* Pharmacology & Therapeutics, 2007. **116**(3): p. 437-348.

25. Kalwat, M.A. and M.H. Cobb, *Mechanisms of the amplifying pathway of insulin secretion in the β cell.* Pharmacology & Therapeutics, 2017. **179**: p. 17-30.

26. Ahren, B., *Islet G protein-coupled receptors as potential targets for treatment of type 2 diabetes.* Nature Review Drug Discovery, 2009. **8**(5): p. 369-385.

27. Komatsu, M., et al., *Mastoparan stimulates exocytosis at a Ca(2+)-independent late site in stimulus-secretion coupling. Studies with the RINm5F beta-cell line.* The Journal of biological chemistry, 1993. **268**(31): p. 23297-23306.

28. Merrins, M.J. and E.L. Stuenkel, *Kinetics of Rab27a-dependent actions on vesicle docking and priming in pancreatic beta-cells.* Journal of Physiology, 2008. **586**(22): p. 5367-5381.

29. Brownlee, M., *The pathobiology of diabetic complications: a unifying mechanism.* Diabetes, 2005. **54**(6): p. 1615-1625.

30. Brownlee, M., *Biochemistry and molecular cell biology of diabetic complications.* Nature, 2001. **414**(6865): p. 813-820.

31. Bettiga, A., et al., *The Modern Western Diet Rich in Advanced Glycation End-Products (AGEs): An Overview of Its Impact on Obesity and Early Progression of Renal Pathology.* Nutrients, 2019. **11**(8): p. 1-19.

32. Williams, B., et al., *Glucose-induced protein kinase C activation regulates vascular permeability factor mRNA expression and peptide production by human vascular smooth muscle cells in vitro.* Diabetes, 1997. **46**(9): p. 1497-1503.

33. Koya, D., et al., *Amelioration of accelerated diabetic mesangial expansion by treatment with a PKC beta inhibitor in diabetic db/db mice, a rodent model for type 2 diabetes.* The Faseb Journal, 2000. **14**(3): p. 439-447.

34. Buse, M.G., *Hexosamines, insulin resistance, and the complications of diabetes: current status.* American journal of physiology. Endocrinology and metabolism, 2006. **290**(1): p. 1-8.

35. Ma, J. and G.W. Hart, *Protein O-GlcNAcylation in diabetes and diabetic complications.* Expert Review of Proteomics, 2013. **10**(4): p. 365-380.

36. Zheng, H., et al., *Protein Modifications as Manifestations of Hyperglycemic Glucotoxicity in Diabetes and Its Complications.* Biochemistry insights, 2016. **9**: p. 1-9.

37. Casqueiro, J., J. Casqueiro, and C. Alves, *Infections in patients with diabetes mellitus: A review of pathogenesis.* Indian journal of endocrinology and metabolism, 2012. **16**(1): p. 27-36.

38. Nitzan, O., et al., *Urinary tract infections in patients with type 2 diabetes mellitus: review of prevalence, diagnosis, and management.* Diabetes, metabolic syndrome and obesity : targets and therapy, 2015. **8**: p. 129-136.

39. Demirel, I., et al., *Activation of the NLRP3 Inflammasome Pathway by Uropathogenic Escherichia coli Is Virulence Factor-Dependent and Influences Colonization of Bladder Epithelial Cells.* Frontiers in Cellular and Infection Microbiology, 2018. **8**: p. 1-14.

40. Foxman, B., *The epidemiology of urinary tract infection.* Nature Review Urology, 2010. **7**(12): p. 653-660.

41. Chromek, M., et al., *The antimicrobial peptide cathelicidin protects the urinary tract against invasive bacterial infection.* Nature Medicine, 2006. **12**(6): p. 636-641.

42. Rowe, T.A. and M. Juthani-Mehta, *Diagnosis and management of urinary tract infection in older adults.* Infectious disease clinics of North America, 2014. **28**(1): p. 75-89.

43. Luthje, P., A.L. Hirschberg, and A. Brauner, *Estrogenic action on innate defense mechanisms in the urinary tract.* Maturitas, 2014. **77**(1): p. 32-36.

44. McLellan, L.K. and D.A. Hunstad, *Urinary Tract Infection: Pathogenesis and Outlook.* Trends in Molecular Medicine, 2016. **22**(11): p. 946-957.

45. Ribic, R., et al., *Effective anti-adhesives of uropathogenic Escherichia coli.* Acta Pharmaceutica, 2018. **68**(1): p. 1-18.

46. Zalewska-Piatek, B.M. and R.J. Piatek, *Alternative treatment approaches of urinary tract infections caused by uropathogenic Escherichia coli strains.* Acta Biochimica Polonica, 2019. **66**(2): p. 129-138.

47. Raeispour, M. and R. Ranjbar, *Antibiotic resistance, virulence factors and genotyping of Uropathogenic Escherichia coli strains.* Antimicrobial Resistance & Infection Control, 2018. **7**(118): p. 1-9.

48. Luthje, P. and A. Brauner, *Novel Strategies in the Prevention and Treatment of Urinary Tract Infections.* Pathogens, 2016. **5**(1): p. 1-14.

49. Ozer, A., et al., *Impaired cytokine expression, neutrophil infiltration and bacterial clearance in response to urinary tract infection in diabetic mice.* Pathogens and Disease, 2015. **73**(3): p. 1-9.

50. Nielubowicz, G.R. and H.L. Mobley, *Host-pathogen interactions in urinary tract infection.* Nature Reviews Urology, 2010. **7**(8): p. 430-441.

51. Lillington, J., S. Geibel, and G. Waksman, *Biogenesis and adhesion of type 1 and P pili.* Biochimica et Biophysica Acta, 2014. **1840**(9): p. 2783-2793.

52. Croxen, M.A. and B.B. Finlay, *Molecular mechanisms of Escherichia coli pathogenicity.* Nature Reviews Microbiology, 2010. **8**(1): p. 26-38.

53. Spencer, J.D., et al., *The innate immune response during urinary tract infection and pyelonephritis.* Pediatric Nephrology, 2014. **29**(7): p. 1139-1149.

54. Duncan, M.J., et al., *Bacterial penetration of bladder epithelium through lipid rafts.* The Journal of Biological Chemistry, 2004. **279**(18): p. 18944-18951.

55. Mulvey, M.A., et al., *Bad bugs and beleaguered bladders: interplay between uropathogenic Escherichia coli and innate host defenses.* Proceedings of the Natlonal Academy of Sci ences USA, 2000. **97**(16): p. 8829-8835.

56. Zhou, G., et al., *Uroplakin Ia is the urothelial receptor for uropathogenic Escherichia coli: evidence from in vitro FimH binding.* Journal of Cell Science, 2001. **114**(22): p. 4095-4103.

57. Eto, D.S., et al., *Clathrin, AP-2, and the NPXY-binding subset of alternate endocytic adaptors facilitate FimH-mediated bacterial invasion of host cells.* Cellular Microbiology, 2008. **10**(12): p. 2553-2567.

58. Eto, D.S., et al., *Integrin-mediated host cell invasion by type 1-piliated uropathogenic Escherichia coli.* PLoS Pathogens, 2007. **3**(7): p. 1-10.

59. Rosen, D.A., et al., *Detection of intracellular bacterial communities in human urinary tract infection.* PLoS Medicine, 2007. **4**(12): p. 1949-1958.

60. Tamadonfar, K.O., et al., *Reaching the End of the Line: Urinary Tract Infections.* Microbiology Spectrum, 2019. **7**(3): p. 1-16.

61. Kostakioti, M., M. Hadjifrangiskou, and S.J. Hultgren, *Bacterial biofilms: development, dispersal, and therapeutic strategies in the dawn of the postantibiotic era.* Cold Spring Harbor Perspectives in Medicine, 2013. **3**(4): p. 1-23.

62. Engelsoy, U., I. Rangel, and I. Demirel, *Impact of Proinflammatory Cytokines on the Virulence of Uropathogenic Escherichia coli.* Frontiers in Microbiology, 2019. **10**: p. 1-12.

63. Luthje, P. and A. Brauner, *Virulence factors of uropathogenic E. coli and their interaction with the host.* Adv Microb Physiol, 2014. **65**: p. 337-72.

64. Eberly, A.R., et al., *Biofilm Formation by Uropathogenic Escherichia coli Is Favored under Oxygen Conditions That Mimic the Bladder Environment.* International Journal of Molecular Sciences, 2017. **18**(10): p. 1-12.

65. Flemming, H.C. and J. Wingender, *The biofilm matrix.* Nature Reviews Microbiology, 2010. **8**(9): p. 623-633.

66. Ching, C., et al., *Innate immunity and urinary tract infection.* Pediatric Nephrology, 2019. **10**(1007): p. 1-10.

67. Hayes, B.W. and S.N. Abraham, *Innate Immune Responses to Bladder Infection.* Microbiology Spectrum, 2016. **4**(6): p. 1-8.

68. Klumpp, D.J., et al., *Uropathogenic Escherichia coli induces extrinsic and intrinsic cascades to initiate urothelial apoptosis.* Infection and immunity, 2006. **74**(9): p. 5106-5113.

69. Mangoni, M.L., A.M. McDermott, and M. Zasloff, *Antimicrobial peptides and wound healing: biological and therapeutic considerations.* Experimental Dermatology, 2016. **25**(3): p. 167-173.

70. Bahar, A.A. and D. Ren, *Antimicrobial peptides.* Pharmaceuticals, 2013. **6**(12): p. 1543-1575.

71. Moravej, H., et al., *Antimicrobial Peptides: Features, Action, and Their Resistance Mechanisms in Bacteria.* Microbial Drug Resistance, 2018. **24**(6): p. 747-767.

72. Sorensen, O.E., N. Borregaard, and A.M. Cole, *Antimicrobial peptides in innate immune responses.* Contributions to Microbiology, 2008. **15**: p. 61-77.

73. Sierra, J.M., et al., *An overview of antimicrobial peptides and the latest advances in their development.* Expert Opinion on Biological Therapy, 2017. **17**(6): p. 663-676.

74. Zasloff, M., *Antimicrobial peptides, innate immunity, and the normally sterile urinary tract.* Journal of the American Society of Nephrology, 2007. **18**(11): p. 2810-2816.

75. Lehmann, J., et al., *Expression of human beta-defensins 1 and 2 in kidneys with chronic bacterial infection.* BMC Infectious Diseases, 2002. **2**(20): p. 1-10.

76. Zasloff, M., *Antimicrobial Peptides of Multicellular Organisms: My Perspective*, in *Antimicrobial Peptides: Basics for Clinical Application*, K. Matsuzaki, Editor. 2019, Springer Singapore: Singapore. p. 3-6.

77. Chromek, M. and A. Brauner, *Antimicrobial mechanisms of the urinary tract.* J Mol Med (Berl), 2008. **86**(1): p. 37-47.

78. Becknell, B. and J.D. Spencer, *A Review of Ribonuclease 7's Structure, Regulation, and Contributions to Host Defense.* International Journl of Molecular Sciences, 2016. **17**(3): p. 1-10.

79. Spencer, J.D., et al., *Ribonuclease 7, an antimicrobial peptide upregulated during infection, contributes to microbial defense of the human urinary tract.* Kidney International, 2013. **83**(4): p. 615-625.

80. Luthje, P., et al., *Estrogen supports urothelial defense mechanisms.* Science Translational Medicine, 2013. **5**(190): p. 1-9.

81. Eichler, T.E., et al., *Insulin and the phosphatidylinositol 3-kinase signaling pathway regulate Ribonuclease 7 expression in the human urinary tract.* Kidney International, 2016. **90**(3): p. 568-579.

82. Kai-Larsen, Y., et al., *Uropathogenic Escherichia coli modulates immune responses and its curli fimbriae interact with the antimicrobial peptide LL-37.* PLoS Pathogens, 2010. **6**(7): p. 1-16.

83. Rios, A.C., et al., *Alternatives to overcoming bacterial resistances: State-of-the-art.* Microbiological Research, 2016. **191**: p. 51-80.

84. Nitzan, O., et al., *Urinary tract infections in patients with type 2 diabetes mellitus: review of prevalence, diagnosis, and management.* Diabetes, Metabolic Syndrome and Obesity, 2015. **8**: p. 129-136.

85. Ozer, A., et al., *Advanced glycation end products facilitate bacterial adherence in urinary tract infection in diabetic mice.* Pathogens and Disease, 2015. **73**(5): p. 1-10.

86. Geerlings, S., et al., *Genital and urinary tract infections in diabetes: Impact of pharmacologically-induced glucosuria.* Diabetes Research and Clinical Practice, 2014. **103**(3): p. 373-381.

87. Vlassara, H. and J. Uribarri, *Advanced glycation end products (AGE) and diabetes: cause, effect, or both?* Curr Diab Rep, 2014. **14**(1): p. 453.

88. Taganna, J., et al., *Glycosylation changes as important factors for the susceptibility to urinary tract infection.* Biochemical Society Transactions, 2011. **39**(1): p. 349-354.

89. Chaudhury, A., et al., *Clinical Review of Antidiabetic Drugs: Implications for Type 2 Diabetes Mellitus Management.* Frontiers in Endocrinology, 2017. **8**: p. 6.

90. Kerru, N., et al., *Current anti-diabetic agents and their molecular targets: A review.* European Journal of Medicinal Chemistry, 2018. **152**: p. 436-488.

91. Seino, S., *Cell signalling in insulin secretion: the molecular targets of ATP, cAMP and sulfonylurea.* Diabetologia, 2012. **55**(8): p. 2096-2108.

92. Chaudhury, A., et al., *Clinical Review of Antidiabetic Drugs: Implications for Type 2 Diabetes Mellitus Management.* Front Endocrinol (Lausanne), 2017. **8**: p. 6.

93. Guardado-Mendoza, R., et al., *The role of nateglinide and repaglinide, derivatives of meglitinide, in the treatment of type 2 diabetes mellitus.* Archives of medical science, 2013. **9**(5): p. 936-943.

94. Viollet, B., et al., *Cellular and molecular mechanisms of metformin: an overview.* Clinical Science 2012. **122**(6): p. 253-270.

95. Kim, W. and J.M. Egan, *The role of incretins in glucose homeostasis and diabetes treatment.* Pharmacological Reviews, 2008. **60**(4): p. 470-512.

96. Kawanami, D., et al., *Incretin-Based Therapies for Diabetic Complications: Basic Mechanisms and Clinical Evidence.* International Journal of Molecular Sciences, 2016. **17**(8): p. 1223.

97. Chikara, G., et al., *A Narrative Review of Potential Future Antidiabetic Drugs: Should We Expect More?* Indian Journal of Clinical Biochemistry, 2018. **33**(2): p. 121-131.

98. Yabe, D., et al., *Sodium glucose co-transporter 2 inhibitor luseogliflozin in the management of type 2 diabetes: a drug safety evaluation.* Expert Opinion on Drug Safety, 2017. **16**(10): p. 1211-1218.

99. Bischoff, H., *The mechanism of alpha-glucosidase inhibition in the management of diabetes.* Clinical and Investigative Medicine, 1995. **18**(4): p. 303-311.

100. Lamos, E.M., L.M. Younk, and S.N. Davis, *Concentrated insulins: the new basal insulins.* Therapeutics and Clinical Risk Management, 2016. **12**: p. 389-400.

101. Thrasher, J., *Pharmacologic Management of Type 2 Diabetes Mellitus: Available Therapies.* American Journal of Medicine, 2017. **130**(6s): p. 4-17.

102. Chaudhury, A., et al., *Clinical Review of Antidiabetic Drugs: Implications for Type 2 Diabetes Mellitus Management.* Frontiers in Endocrinology, 2017. **8**(6): p. 1-12.

103. Mazzariol, A., A. Bazaj, and G. Cornaglia, *Multi-drug-resistant Gram-negative bacteria causing urinary tract infections: a review.* Journal of Chemotherapy, 2017. **29**(1): p. 2-9.

104. Naber, K.G. and F.M.E. Wagenlehner, *Novel Antibiotics in the Treatment of Urinary Tract Infections.* European Urology Focus, 2019. **5**(1): p. 10-12.

105. Frieri, M., K. Kumar, and A. Boutin, *Antibiotic resistance.* Journal of Infection and Public Health, 2017. **10**(4): p. 369-378.

106. Poirel, L., et al., *Antimicrobial Resistance in Escherichia coli.* Microbiology Spectrum, 2018. **6**(4): p. 1-27.

107. Zowawi, H.M., et al., *The emerging threat of multidrug-resistant Gram-negative bacteria in urology.* Nature Reviews Urology, 2015. **12**(10): p. 570-584.

108. Karaman, E., et al., *The use of herbal supplements by individuals with diabetes mellitus.* Journal of the Pakistan Medical Association, 2018. **68**(4): p. 587-594.

109. Lankatillake, C., T. Huynh, and D.A. Dias, *Understanding glycaemic control and current approaches for screening antidiabetic natural products from evidence-based medicinal plants.* Plant Methods, 2019. **15**(105): p. 1-35.

110. Kooti, W., et al., *The role of medicinal plants in the treatment of diabetes: a systematic review.* Electronic physician, 2016. **8**(1): p. 1832-1842.

111. Girault, L., C. Bustillos, and R. Alcocer, *Kallawaya: Curanderos Itinerantes de los andes, Investigación sobre prácticas medicinales y mágicas*, in *Kallawaya: curanderos itinerantes de los Andes, investigación sobre prácticas medicinales y mágicas*. 1987, Unicef.

112. Yilmaz, Z., et al., *Supplements for Diabetes Mellitus: A Review of the Literature.* Journal of Pharmacy Practice, 2017. **30**(6): p. 631-638.

113. Yeh, G.Y., et al., *Systematic review of herbs and dietary supplements for glycemic control in diabetes.* Diabetes Care, 2003. **26**(4): p. 1277-1294.

114. Rodrigues, E.L., et al., *Nutraceutical and Medicinal Potential of the Morus Species in Metabolic Dysfunctions.* International Journal of Molecular Sciences, 2019. **20**(2): p. 1-16.

115. Bindu, J. and R.T. Narendhirakannan, *Role of medicinal plants in the management of diabetes mellitus: a review.* 3 Biotech, 2019. **9**(4): p. 1-17.

116. Huang, F.Y., et al., *Dietary ginger as a traditional therapy for blood sugar control in patients with type 2 diabetes mellitus: A systematic review and meta-analysis.* Medicine, 2019. **98**(13): p. 1-13.

117. Huyen, V.T., et al., *Gynostemma pentaphyllum Tea Improves Insulin Sensitivity in Type 2 Diabetic Patients.* Journal of Nutrition and Metabolism, 2013. **2013**: p. 1-7.

118. Norberg, A., et al., *A novel insulin-releasing substance, phanoside, from the plant Gynostemma pentaphyllum.* Journal of Biological Chemistry, 2004. **279**(40): p. 41361-41367.

119. Lundqvist, L.C.E., et al., *Profiling and activity screening of Dammarane-type triterpen saponins from Gynostemma pentaphyllum with glucose-dependent insulin secretory activity.* Scientific Reports, 2019. **9**(1): p. 1-10.

120. Yassin, K., et al., *Herbal extract of gynostemma pentaphyllum decreases hepatic glucose output in type 2 diabetic goto-kakizaki rats.* International Journal of Biomedical Science, 2011. **7**(2): p. 131-136.

121. Salehi, B., et al., *Antidiabetic Potential of Medicinal Plants and Their Active Components.* Biomolecules, 2019. **9**(10): p. 1-121.

122. Naseri, R., et al., *Anthocyanins in the Management of Metabolic Syndrome: A Pharmacological and Biopharmaceutical Review.* Front Pharmacol, 2018. **9**: p. 1-19.

123. Beydokthi, S.S., et al., *Traditionally used medicinal plants against uncomplicated urinary tract infections: Hexadecyl coumaric acid ester from the rhizomes of Agropyron repens (L.) P. Beauv. with antiadhesive activity against uropathogenic E. coli.* Fitoterapia, 2017. **117**: p. 22-27.

124. Lagha, R., et al., *Antibacterial and Biofilm Inhibitory Activity of Medicinal Plant Essential Oils Against Escherichia coli Isolated from UTI Patients.* Molecules 2019. **24**(6): p. 1-12.

125. Devi, A.S. and J. Rajkumar, *In vitro antibacterial activity and stability of Avicennia marina against urinary tract infection pathogens at different parameters.* Pak J Biol Sci, 2013. **16**(19): p. 1034-1039.

126. Rafsanjany, N., et al., *Antiadhesion as a functional concept for protection against uropathogenic Escherichia coli: in vitro studies with traditionally used plants with antiadhesive activity against uropathognic Escherichia coli.* Journal of Ethnopharmacol, 2013. **145**(2): p. 591-597.

127. Peron, G., et al., *Antiadhesive Activity and Metabolomics Analysis of Rat Urine after Cranberry (Vaccinium macrocarpon Aiton) Administration.* Journal of Agricultural and Food Chemistry, 2017. **65**(28): p. 5657-5667.

128. Singh, I., L.K. Gautam, and I.R. Kaur, *Effect of oral cranberry extract (standardized proanthocyanidin-A) in patients with recurrent UTI by pathogenic E. coli: a randomized placebo-controlled clinical research study.* International Urology and Nephrology, 2016. **48**(9): p. 1379-1386.

129. Beerepoot, M.A., et al., *Cranberries vs antibiotics to prevent urinary tract infections: a randomized double-blind noninferiority trial in premenopausal women.* Archives of Internal Medicine, 2011. **171**(14): p. 1270-1278.

130. Altarac, S. and D. Papeš, *Use of d-mannose in prophylaxis of recurrent urinary tract infections (UTIs) in women.* BJU International, 2014. **113**(1): p. 9-10.

131. Genovese, C., S. Davinelli, and K. Mangano, *Effects of a new combination of plant extracts plus d-mannose for the management of uncomplicated recurrent urinary tract infections.* Journal of Chemotherapy, 2018. **30**(2): p. 107-114.

132. Sarshar, S., et al., *Antiadhesive hydroalcoholic extract from Apium graveolens fruits prevents bladder and kidney infection against uropathogenic E. coli.* Fitoterapia, 2018. **127**: p. 237-244.

133. Mohanty, S., et al., *Extract of Clinopodium bolivianum protects against E. coli invasion of uroepithelial cells.* Journal of Ethnopharmacology, 2017. **198**: p. 214-220.

134. Vollmerhausen, T.L., et al., *Decoctions from Citrus reticulata Blanco seeds protect the uroepithelium against Escherichia coli invasion.* Journal of Ethnopharmacology, 2013. **150**(2): p. 770-774.

135. Wojnicz, D., et al., *Medicinal plants extracts affect virulence factors expression and biofilm formation by the uropathogenic Escherichia coli.* Urological Research, 2012. **40**(6): p. 683-697.

136. Kalra, E.K., *Nutraceutical--definition and introduction.* American Asociation of Pharmaceutical Scientists 2003. **5**(3): p. 27-28.

137. Santini, A., G.C. Tenore, and E. Novellino, *Nutraceuticals: A paradigm of proactive medicine.* Eur J Pharm Sci, 2017. **96**: p. 53-61.

138. Rios-Hoyo, A., et al., *Prehispanic Functional Foods and Nutraceuticals in the Treatment of Dyslipidemia Associated to Cardiovascular Disease: a Mini-Review.* International journal for vitamin and nutrition research, 2017. **87**(1-2): p. 58-98.

139. Santini, A., et al., *Nutraceuticals: opening the debate for a regulatory framework.* British Pharmacological Society, 2018. **84**(4): p. 659-672.

140. Daliu, P. and A. Santini, *From pharmaceuticals to nutraceuticals: bridging disease prevention and management.* Expert Review of Clinical Pharmacology, 2019. **12**(1): p. 1-7.

141. Beidokhti, M.N. and A.K. Jager, *Review of antidiabetic fruits, vegetables, beverages, oils and spices commonly consumed in the diet.* Journal of Ethnopharmacology, 2017. **201**: p. 26-41.

142. Cicero, A.F.G., et al., *Nutraceutical Effects on Glucose and Lipid Metabolism in Patients with Impaired Fasting Glucose: A Pilot, Double-Blind, Placebo-Controlled, Randomized Clinical Trial on a Combined Product.* High Blood Press Cardiovasc Prev, 2017. **24**(3): p. 283-288.

143. Lee, T. and J.J. Dugoua, *Nutritional supplements and their effect on glucose control.* Current Diabetes Reports, 2011. **11**(2): p. 142-148.

144. Campos, D., et al., *Bioactive Potential of Andean Fruits, Seeds, and Tubers.* Advances in Food and Nutrition Research, 2018. **84**: p. 287-343.

145. Caselato-Sousa, V.M. and J. Amaya-Farfan, *State of knowledge on amaranth grain: a comprehensive review.* Journal of Food Science, 2012. **77**(4): p. 93-104.

146. Sawangjaroen, N. and K. Sawangjaroen, *The effects of extracts from anti-diarrheic Thai medicinal plants on the in vitro growth of the intestinal protozoa parasite: Blastocystis hominis.* Journal of Ethnopharmacology, 2005. **98**(1-2): p. 67-72.

147. Girija, K., et al., *Anti-diabetic and anti-cholesterolemic activity of methanol extracts of three species of Amaranthus.* Asian Pacific journal of tropical biomedicine, 2011. **1**(2): p. 133-138.

148. Lamothe, L.M., et al., *Quinoa (Chenopodium quinoa W.) and amaranth (Amaranthus caudatus L.) provide dietary fibres high in pectic substances and xyloglucans.* Food Chemistry, 2015. **167**: p. 490-496.

149. Kumar, A., et al., *In Vitro α-Amylase Inhibition and Antioxidant Activities of Methanolic Extract of Amaranthus Caudatus Linn.* Oman Medical Journal, 2011. **26**(3): p. 166-170.

150. Vega-Galvez, A., et al., *Nutrition facts and functional potential of quinoa (Chenopodium quinoa willd.), an ancient Andean grain: a review.* Journal of the Science of Food and Agriculture, 2010. **90**(15): p. 2541-2547.

151. Gawlik-Dziki, U., et al., *Antioxidant and anticancer activities of Chenopodium quinoa leaves extracts - in vitro study.* Food and Chemical Toxicology, 2013. **57**: p. 154-160.

152. Filho, A.M., et al., *Quinoa: Nutritional, functional, and antinutritional aspects.* Critical Reviews in Food Science and Nutrition., 2017. **57**(8): p. 1618-1630.

153. Repo-Carrasco, R., C. Espinoza, and S.E. Jacobsen, *Nutritional value and use of the Andean crops quinoa (Chenopodium quinoa) and kaniwa (Chenopodium pallidicaule).* Food Reviews International, 2003. **19**(1-2): p. 179-189.

154. Repo-Carrasco-Valencia, R., et al., *Chemical and functional characterization of Kaniwa (Chenopodium pallidicaule) grain, extrudate and bran.* Plant Foods for Human Nutrition, 2009. **64**(2): p. 94-101.

155. Repo-Carrasco-Valencia, R., et al., *Flavonoids and other phenolic compounds in Andean indigenous grains: Quinoa (Chenopodium quinoa), kaniwa (Chenopodium pallidicaule) and kiwicha (Amaranthus caudatus).* Food Chemistry, 2010. **120**(1): p. 128-133.

156. Penarrieta, J.M., et al., *Total antioxidant capacity and content of flavonoids and other phenolic compounds in canihua (Chenopodium pallidicaule): an Andean pseudocereal.* Molecular Nutrition & Food Research, 2008. **52**(6): p. 708-717.

157. Rastrelli, L., et al., *Constituents of Chenopodium pallidicaule (Canihua) seeds: Isolation and characterization of new triterpene saponins.* Journal of Agricultural and Food Chemistry, 1996. **44**(11): p. 3528-3533.

158. Rastrelli, L., et al., *Studies on the Constituents of Chenopodium Pallidicaule (Canihua) Seeds - Isolation and Characterization of 2 New Flavonol Glycosides.* Journal of Agricultural and Food Chemistry, 1995. **43**(8): p. 2020-2024.

159. Schoeneberger, H., et al., *Composition and protein quality of Lupinus mutabilis.* Journal of Nutrition, 1982. **112**(1): p. 70-76.

160. Ortega Duarte, A., et al., *Lupinus mutabilis (chocho) water intoxication.* Medicina Clinica, 2013. **140**(1): p. 43-44.

161. Fornasini, M., et al., *Hypoglycemic effect of Lupinus mutabilis in healthy volunteers and subjects with dysglycemia.* Nutrcion Hospitalaria, 2012. **27**(2): p. 425-433.

162. Genta, S., et al., *Yacon syrup: Beneficial effects on obesity and insulin resistance in humans.* Clinical Nutrition, 2009. **28**(2): p. 182-187.

163. Lachman, J., et al., *Saccharides of yacon Smallanthus sonchifolius (Poepp. et Endl.) H. Robinson tubers and rhizomes and factors affecting their content.* Plant Soil and Environment, 2004. **50**(9): p. 383-390.

164. Genta, S.B., et al., *Hypoglycemic activity of leaf organic extracts from Smallanthus sonchifolius: Constituents of the most active fractions.* Chem Biol Interact, 2010. **185**(2): p. 143-52.

165. Aybar, M.J., et al., *Hypoglycemic effect of the water extract of Smallantus sonchifolius (yacon) leaves in normal and diabetic rats.* Journal of Ethnopharmacology, 2001. **74**(2): p. 125-132.

166. Serra-Barcellona, C., et al., *Enhydrin Regulates Postprandial Hyperglycemia in Diabetic Rats by Inhibition of alpha-Glucosidase Activity.* Plant foods for human nutrition, 2017. **72**(2): p. 156-160.

167. Habib, N.C., et al., *Yacon roots (Smallanthus sonchifolius) improve oxidative stress in diabetic rats.* Pharmaceutical biology, 2015. **53**(8): p. 1183-1193.

168. Hildreth, J., et al., *Standard operating procedure for the collection and preparation of voucher plant specimens for use in the nutraceutical industry.* Analytical and Bioanalytical Chemistry, 2007. **389**(1): p. 13-17.

169. Gonzales, E., et al., *Gastric cytoprotection of bolivian medicinal plants.* J Ethnopharmacol, 2000. **70**(3): p. 329-33.

170. Harborne, J., *Phytochemical Methods: A Guide to Modern Techniquesof Plant Analysis*. 1998, London: Chapman and Hall.

171. Oecd, *Test No. 423: Acute Oral toxicity - Acute Toxic Class Method*. OECD Publishing.

172. Ostenson, C.G., et al., *Impaired gene and protein expression of exocytotic soluble N-ethylmaleimide attachment protein receptor complex proteins in pancreatic islets of type 2 diabetic patients.* Diabetes, 2006. **55**(2): p. 435-440.

173. Domingues, N., et al., *Therapeutic properties of VO(dmpp)2 as assessed by in vitro and in vivo studies in type 2 diabetic GK rats.* Journal of Inorganic Biochemistry, 2014. **131**: p. 115-122.

174. Lokman, F.E., et al., *Antidiabetic Effect of Oral Borapetol B Compound, Isolated from the Plant Tinospora crispa, by Stimulating Insulin Release.* Evidence-based complementary and alternative medicine, 2013. **2013**: p. 1-7.

175. Hoa, N.K., et al., *Screening of the hypoglycemic effect of eight Vietnamese herbal drugs.* Methods and Findings in Experimental and Clinical Pharmacology, 2009. **31**(3): p. 165-169.

176. Stumvoll, M., et al., *Use of the oral glucose tolerance test to assess insulin release and insulin sensitivity.* Diabetes Care, 2000. **23**(3): p. 295-301.

177. Bartoli, E., G.P. Fra, and G.P. Carnevale Schianca, *The oral glucose tolerance test (OGTT) revisited.* European Journal of Internal Medicine, 2011. **22**(1): p. 8-12.

178. Herbert, V., et al., *Coated charcoal immunoassay of insulin.* Journal of Clinical Endocrinology and Metabolism, 1965. **25**(10): p. 1375-1384.

179. Lokman, E.F., et al., *Evaluation of Antidiabetic Effects of the Traditional Medicinal Plant Gynostemma pentaphyllum and the Possible Mechanisms of Insulin Release.* Evidence-based Complementary and Alternative Medicine, 2015. **2015**: p. 1-7.

180. Oecd, *Test No. 407: Repeated Dose 28-day Oral Toxicity Study in Rodents*. OECD Publishing.

181. Li, D.-S., et al., *A protocol for islet isolation from mouse pancreas.* Nature Protocols, 2009. **4**: p. 1649-1652.

182. Hoa, N.K., et al., *The possible mechanisms by which phanoside stimulates insulin secretion from rat islets.* Journal of Endocrinology, 2007. **192**(2): p. 389-394.

183. Mosmann, T., *Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays.* Journal of Immunological Methods, 1983. **65**(1-2): p. 55-63.

184. Catrina, S.-B., et al., *A cytotoxic, apoptotic, low -molecular weight factor from pineal gland.* Life Sciences, 1999. **65**(10): p. 1047-1057.

185. Scudiero, D.A., et al., *Evaluation of a soluble tetrazolium/formazan assay for cell growth and drug sensitivity in culture using human and other tumor cell lines.* Cancer Research, 1988. **48**(17): p. 4827-4833.

186. Ngamwongsatit, P., et al., *WST-1-based cell cytotoxicity assay as a substitute for MTT-based assay for rapid detection of toxigenic Bacillus species using CHO cell line.* Journal of Microbiological Methods, 2008. **73**(3): p. 211-215.

187. Doyle, M.E. and J.M. Egan, *Pharmacological agents that directly modulate insulin secretion.* Pharmacological Reviews, 2003. **55**(1): p. 105-131.

188. Farret, A., et al., *Pharmacological interventions that directly stimulate or modulate insulin secretion from pancreatic beta-cell: implications for the treatment of type 2 diabetes.* Fundamental & Clinical Pharmacology, 2005. **19**(6): p. 647-656.

189. Karbalaei, N., A. Noorafshan, and E. Hoshmandi, *Impaired glucose-stimulated insulin secretion and reduced beta-cell mass in pancreatic islets of hyperthyroid rats.* Experimental Physiology, 2016. **101**(8): p. 1114-1127.

190. Pelletier, J., et al., *In vitro effects of bis(1,2-dimethyl-3-hydroxy-4-pyridinonato) oxidovanadium(IV), or VO(dmpp)2, on insulin secretion in pancreatic islets of type 2 diabetic Goto-Kakizaki rats.* Journal of Inorganic Biochemistry, 2016. **154**: p. 29-34.

191. Lochner, A. and J.A. Moolman, *The many faces of H89: a review.* Cardiovascular Drug Reviews, 2006. **24**(3-4): p. 261-274.

192. Wan, Q.-F., et al., *Protein Kinase Activation Increases Insulin Secretion by Sensitizing the Secretory Machinery to Ca(2+).* The Journal of General Physiology, 2004. **124**(6): p. 653-662.

193. Kobayashi, E., et al., *Calphostin C (UCN-1028C), a novel microbial compound, is a highly potent and specific inhibitor of protein kinase C.* Biochemical and Biophysical Research Communications, 1989. **159**(2): p. 548-553.

194. Domingues, N., et al., *Therapeutic properties of VO(dmpp)2 as assessed by in vitro and in vivo studies in type 2 diabetic GK rats.* Journal of Inorganic Biochemistry, 2014. **131**: p. 115-22.

195. Søraas, A., et al., *Extended-spectrum beta-lactamase-producing bacteria are not detected in supragingival plaque samples from human fecal carriers of ESBL-producing Enterobacteriaceae.* Journal of oral Microbiology, 2014. **6**: p. 1-7.

196. Minogue, T.D., et al., *Complete Genome Assembly of Escherichia coli ATCC 25922, a Serotype O6 Reference Strain.* Genome Announcements, 2014. **2**(5): p. 14.

197. Andrews, J.M., *Determination of minimum inhibitory concentrations.* Journal of Antimicrobial Chemotherapy, 2001. **48** (1): p. 5-16.

198. Lüthje, P., et al., *Gynostemma pentaphyllum exhibits anti-inflammatory properties and modulates antimicrobial peptide expression in the urinary bladder.* Journal of Functional Foods, 2015. **17**: p. 283-292.

199. Felicetti, F., et al., *Caveolin-1 tumor-promoting role in human melanoma.* International Journal of Cancer, 2009. **125**(7): p. 1514-1522.

200. Xu, Y., et al., *Proinflammatory phenotype and increased caveolin-1 in alveolar macrophages with silenced CFTR mRNA.* PloS one, 2010. **5**(6): p. e11004-e11004.

201. Dayalan Naidu, S., et al., *Transcription factors NRF2 and HSF1 have opposing functions in autophagy.* 2017. **7**(1): p. 11023.

202. Wehkamp, J., et al., *NF-kappaB- and AP-1-mediated induction of human beta defensin-2 in intestinal epithelial cells by Escherichia coli Nissle 1917: a novel effect of a probiotic bacterium.* Infection and immunity, 2004. **72**(10): p. 5750-5758.

203. Spencer, J.D., et al., *Ribonuclease 7 is a potent antimicrobial peptide within the human urinary tract.* Kidney International, 2011. **80**(2): p. 174-180.

204. Park, H.J., et al., *Disulfiram deregulates HIF-alpha subunits and blunts tumor adaptation to hypoxia in hepatoma cells.* Acta Pharmacol Sin, 2013. **34**(9): p. 1208-16.

205. Donaldson, J.G., *Immunofluorescence Staining.* Curr Protoc Cell Biol, 2015. **69**: p. 4.3.1-7.

206. Shukla, S.K. and T.S. Rao, *An Improved Crystal Violet Assay for Biofilm Quantification in 96-Well Microtitre Plate.* bioRxiv, 2017: p. 100214.

207. Sultana, B., F. Anwar, and M. Ashraf, *Effect of extraction solvent/technique on the antioxidant activity of selected medicinal plant extracts.* Molecules, 2009. **14**(6): p. 2167-2180.

208. Lapornik, B., M. Prošek, and A. Golc Wondra, *Comparison of extracts prepared from plant by-products using different solvents and extraction time.* Journal of Food Engineering, 2005. **71**(2): p. 214-222.

209. Conforti, F., et al., *In Vitro antioxidant effect and inhibition of alpha-amylase of two varieties of Amaranthus caudatus seeds.* Biol Pharm Bull, 2005. **28**(6): p. 1098-102.

210. Kumar, A., et al., *In Vitro alpha-Amylase Inhibition and Antioxidant Activities of Methanolic Extract of Amaranthus Caudatus Linn.* Oman Med J, 2011. **26**(3): p. 166-70.

211. Fornasini, M., et al., *Hypoglycemic effect of Lupinus mutabilis in healthy volunteers and subjects with dysglycemia.* Nutricion Hospitalaria, 2012. **27**(2): p. 425-433.

212. Baldeon, M.E., et al., *Hypoglycemic effect of cooked Lupinus mutabilis and its purified alkaloids in subjects with type-2 diabetes.* Nutricion Hospitalaria, 2012. **27**(4): p. 1261-1266.

213. Bahadoran, Z., P. Mirmiran, and F. Azizi, *Dietary polyphenols as potential nutraceuticals in management of diabetes: a review.* J Diabetes Metab Disord, 2013. **12**(1): p. 43.

214. Pasko, P., et al., *Effect of Diet Supplemented with Quinoa Seeds on Oxidative Status in Plasma and Selected Tissues of High Fructose-Fed Rats.* Plant Foods for Human Nutrition, 2010. **65**(2): p. 146-151.

215. Conforti, F., et al., *In Vitro antioxidant effect and inhibition of alpha-amylase of two varieties of Amaranthus caudatus seeds.* Biological & Pharmaceutical Bulletin, 2005. **28**(6): p. 1098-1102.

216. Girija, K., et al., *Anti-diabetic and anti-cholesterolemic activity of methanol extracts of three species of Amaranthus.* Asian Pac J Trop Biomed, 2011. **1**(2): p. 133-8.

217. Montero-Quintero, K.C., et al., *[Effect of consumption of bread with amaranth (Amaranthus dubius Mart. ex Thell.) on glycemic response and biochemical parameters in Sprague dawley rats].* Nutricion Hospitalaria, 2014. **31**(1): p. 313-320.

218. Rahmatullah, M., et al., *Antihyperglycaemic and antinociceptive activity evaluation of methanolic extract of whole plant of Amaranthus tricolour L. (Amaranthaceae).* African Journal of Traditional, Complementary and Alternative Medicines 2013. **10**(5): p. 408-411.

219. Ashok Kumar, B.S., et al., *Antidiabetic, antihyperlipidemic and antioxidant activities of methanolic extract of Amaranthus viridis Linn in alloxan induced diabetic rats.* Experimental and Toxicologic Pathology, 2012. **64**(1-2): p. 75-79.

220. Krishnamurthy, G., et al., *Antihyperglycemic and hypolipidemic activity of methanolic extract of Amaranthus viridis leaves in experimental diabetes.* Indian Journal of Pharmacology, 2011. **43**(4): p. 450-454.

221. Sangameswaran, B. and B. Jayakar, *Anti-diabetic, anti-hyperlipidemic and spermatogenic effects of Amaranthus spinosus Linn. on streptozotocin-induced diabetic rats.* Journal of nNtural Medicines, 2008. **62**(1): p. 79-82.

222. Paśko, P., et al., *Anthocyanins, total polyphenols and antioxidant activity in amaranth and quinoa seeds and sprouts during their growth.* Food Chemistry, 2009. **115**(3): p. 994-998.

223. Venskutonis, P.R. and P. Kraujalis, *Nutritional Components of Amaranth Seeds and Vegetables: A Review on Composition, Properties, and Uses.* Comprehensive Reviews in Food Science and Food Safety, 2013. **12**(4): p. 381-412.

224. Hoa, N.K., et al., *Insulin secretion is stimulated by ethanol extract of Anemarrhena asphodeloides in isolated islet of healthy Wistar and diabetic Goto-Kakizaki Rats.* Experimental and Clinical Endocrinology & Diabetes 2004. **112**(9): p. 520-525.

225. Barker, C.J. and P.O. Berggren, *New horizons in cellular regulation by inositol polyphosphates: insights from the pancreatic beta-cell.* Pharmacological Reviews, 2013. **65**(2): p. 641-669.

226. Ahren, B., *Islet G protein-coupled receptors as potential targets for treatment of type 2 diabetes.* Nat Rev Drug Discov, 2009. **8**(5): p. 369-385.

227. Jacobsen, S.-E.M., Angel, *El tarwi (Lupinus mutabilis Sweet.) y sus parientes silvestres*, in *Botánica Económica de los Andes Centrales*. 2006, Universidad Mayor de San Andrés. p. 458 - 482.

228. Dini, I., O. Schettino, and A. Dini, *Studies on the constituents of Lupinus mutabilis (Fabaceae). Isolation and characterization of two new isoflavonoid derivatives.* Journal of Agricultural and Food Chemistry, 1998. **46**(12): p. 5089-5092.

229. Garcia Lopez, P.M., et al., *Quinolizidine alkaloids isolated from Lupinus species enhance insulin secretion.* European Journal of Pharmacology, 2004. **504**(1-2): p. 139-142.

230. Wiedemann, M., et al., *Lupanine Improves Glucose Homeostasis by Influencing KATP Channels and Insulin Gene Expression.* Molecules, 2015. **20**(10): p. 19085-100.

231. Paolisso, G., et al., *Plasma glucose lowering effect of sparteine sulphate infusion in non-insulin dependent (type 2) diabetic subjects.* European Journal of Clinical Pharmacology, 1988. **34**(3): p. 227-232.

232. Bobkiewicz-Kozlowska, T., et al., *Hypoglycaemic effect of quinolizidine alkaloids--lupanine and 2-thionosparteine on non-diabetic and streptozotocin-induced diabetic rats.* European Journal of Pharmacology, 2007. **565**(3): p. 240-244.

233. Chirinos-Arias, M.C., *Andean Lupin (Lupinus mutabilis Sweet) a plant with nutraceutical and medicinal potential.* Revista Bio Ciencias, 2015. **3**(3): p. 163-172.

234. Kroc, M., et al., *Quantitative and qualitative analysis of alkaloids composition in the seeds of a white lupin (Lupinus albus L.) collection.* Genetic Resources and Crop Evolution, 2016. **64**(8): p. 1853-1860.

235. Schoeneberger, H., et al., *Composition and protein quality of Lupinus mutabilis.* J Nutr, 1982. **112**(1): p. 70-6.

236. de Lastours, V. and B. Foxman, *Urinary tract infection in diabetes: epidemiologic considerations.* Current Infectious Disease Reports, 2014. **16**(1): p. 389-395.

237. Noumedem, J.A., et al., *Antibacterial activities of the methanol extracts of ten Cameroonian vegetables against Gram-negative multidrug-resistant bacteria.* BMC Complementary and Alternative Medicine, 2013. **13**: p. 1-26.

238. Saeidi, S., et al., *Antibacterial Activity of Some Plant Extracts Against Extended- Spectrum Beta-Lactamase Producing Escherichia coli Isolates.* Jundishapur journal of microbiology, 2015. **8**(2): p. e15434-e15434.

239. Vogel, N.W., et al., *Assessment of the antimicrobial effect of three plants used for therapy of community-acquired urinary tract infection in Rio Grande do Sul (Brazil).* Journal of Ethnopharmacology, 2011. **137**(3): p. 1334-1336.

240. Luthje, P., D.N. Dzung, and A. Brauner, *Lactuca indica extract interferes with uroepithelial infection by Escherichia coli.* Journal of Ethnopharmacology, 2011. **135**(3): p. 672-677.

241. Shen, X.F., et al., *Dietary flavonoid luteolin attenuates uropathogenic Escherichia. Coli invasion of the urinary bladder.* Biofactors, 2016. **42**(6): p. 674-685.

242. Shen, X.-f., et al., *Luteolin decreases the attachment, invasion and cytotoxicity of UPEC in bladder epithelial cells and inhibits UPEC biofilm formation.* Food and Chemical Toxicology, 2014. **72**: p. 204-211.

243. Wu, X.-R., et al., *Uroplakins in urothelial biology, function, and disease.* Kidney international, 2009. **75**(11): p. 1153-1165.

244. Bower, J.M., D.S. Eto, and M.A. Mulvey, *Covert operations of uropathogenic Escherichia coli within the urinary tract.* Traffic, 2005. **6**(1): p. 18-31.

245. Zhou, G., et al., *Uroplakin Ia is the urothelial receptor for uropathogenic Escherichia coli: evidence from in vitro FimH binding.* J Cell Sci, 2001. **114**(Pt 22): p. 4095-103.

246. Mulvey, M.A., J.D. Schilling, and S.J. Hultgren, *Establishment of a persistent Escherichia coli reservoir during the acute phase of a bladder infection.* Infection and immunity, 2001. **69**(7): p. 4572-4579.

247. O'Brien, V.P., et al., *Drug and Vaccine Development for the Treatment and Prevention of Urinary Tract Infections.* Microbiology Spectrum, 2016. **4**(1): p. 1-42.

248. Jarvis, C., et al., *Antivirulence Isoquinolone Mannosides: Optimization of the Biaryl Aglycone for FimH Lectin Binding Affinity and Efficacy in the Treatment of Chronic UTI.* ChemMedChem, 2016. **11**(4): p. 367-373.

249. Gandee, L., et al., *The efficacy of immediate versus delayed antibiotic administration on bacterial growth and biofilm production of selected strains of uropathogenic Escherichia coli and Pseudomonas aeruginosa.* International Brazilian Journal of Urology, 2015. **41**(1): p. 67-77.

250. Zafriri, D., et al., *Inhibitory activity of cranberry juice on adherence of type 1 and type P fimbriated Escherichia coli to eucaryotic cells.* Antimicrobial Agents and Chemotherapy, 1989. **33**(1): p. 92-98.