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Tropical and Subtropical Leguminosae as Source of Isoflavonoids: literature analysis

by

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Abstract

This bachelor thesis is literary review describing contemporary status of knowledge about quantitative and qualitative content of isoflavonoids in the tropical and subtropical useful plants of the family Leguminosae. Data were acquired from the scientific databases such as Web of Science (WOS) and similar processed literary sources which were published up to the present day. As a result, the table of useful legumes is presented comprising information, whether individual plants contain isoflavonoids or not. From the 170 chosen tropical and subtropical useful plants of the family Leguminosae, 45 of them contain isoflavonoids, 2 of them homoisoflavonoids and 1 plant from the list contain isoflavanquinone. The results showed that still more than one hundred of chosen plants remain to examination. Moreover, a number of this species e.g. *Tamarindus indica* or *Lablab purpureus* is of important economic use.

Key words: isoflavonoids, pulses, useful tropical legumes

Abstrakt

Tato bakalářská práce je literární rešerší popisující kvalitativní a kvantitativní složení isoflavonoidů v tropických a subtropických užitkových rostlinách čeledi *Leguminosae.* Informace byly získány z článků a z databází jakou je Web of Science (WOS) a podobně zpracovaných literárních zdrojů, které byly dodnes publikovány. Výsledkem je tabulka leguminóz, která obsahuje informace, zda-li jednotlivé rostliny obsahují isoflavonoidy či nikoli. Ze 170 vybraných tropických a subtropických užitkových rostliny čeledi *Leguminosae*, 45 z nich obsahuje isoflavonoidy, 2 z nich homoisoflavonoidy a 1 rostlina ze seznamu obsahuje isoflavanchinon. Výsledky ukazují, že stále více jak sto z vybraných rostlin zbývá k prokoumání. Navíc některé z nich mají důležité ekonomické využití, např. *Tamarindus indica* nebo *Lablab purpureus*.

Klíčová slova: isoflavonoidy, luskoviny, užitkové tropické luštěniny

Certification

I, Jana Hummelová, declare that this Bachelor thesis, submitted in partial fulfillment of the requirements for the degree of bachelor (Bc.), in the Institute of Tropics and Subtropics of the Czech University of Life Sciences, Prague, is wholly my own work unless otherwise referenced or acknowledged.

Prague, 17th March 2008

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Jana Hummelová

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Content

Foreword

Isoflavonoids are secondary metabolites of plants, serving as mediators in a lot of interactions between plant and its surround. Thanks to its biological activities, isoflavonoids belong to the pharmacologically important matters, attracting attention of human and vet medicine. From the chemical point of view we speak about heterocyclic phenols, which have got an estrogen-like structure. For the first time, isoflavonoids were discovered as antinutrition matters (cause problems with reproduction) to animals, which had high content of plants with these matters in its diet.

Generally, isoflavonoids have many interesting properties, in human and vet medicine, which are e.g. interaction with estrogen receptors or inhibition of some important enzymes (tyrosine), which are connected with cell growth and proliferation. These properties express mainly as anti-inflammatory, antibacterial and antiviral activity, interferencing of the cholesterol level in blood, lowering of osteoporosis and cardiovascular risks. A lot of science studies both on animals and tissue cultures show that isoflavonoids inhibit the growth of some tumor lines. In the several latest years, the researches focused on the possibilities of treatment and prevention of mamma and prostrate tumor has been provided.

There are the gene centers of many plants species in tropical and subtropical areas. These large, but only in a small-scale explored, biodiversity still offered discovering of plants, which contain biologically active matters with broad uses in nutrition, pharmacy or food industry. Isoflavonoids have been detected already in some tropical and subtropical Leguminosae plants, but still there are many plants from this family, which come from these areas and which are commonly use as food for human and animals, but qualitative and quantitative analysis of isoflavonoids have not been provided yet.

1 Introduction

The Leguminosae is one of the largest families of flowering plants with 19,325 species classified into around 727 genera. Only families Asteraceae and Orchidaceae are more species-rich (Veitch, 2007).

Leguminosae are a significant component of nearly all terrestrial biomes, on all continents (except Antarctica). The species within the family range from dwarf herbs of arctic and alpine vegetation through shrubs to massive trees and lianes of tropical forest. They are second only to the grasses in economic value (Harborne *et al.*, 1971). They are important to agriculture because they fix atmospheric nitrogen and that is why they have a high feeding value (Bogdan, 1977).

1.1 Botany

1.1.1 Scientific classification

Table 1: Systematic submission of the family Leguminosae

Kingdom	Plantae
Division	Magnoliophyta
Class	Magnoliopsida
Order	Fabales
Family	Leguminosae

Scientific classification of Leguminosae is very complicated, but for the purpose of this thesis we used classification, which is shown in Table 1 [adopted from Lewis *et al.* (2005)]. According to the nomenclature, the Leguminosae family is often taken into account as three separate families: Papilionaceae, Caesalpiniaceae and Mimosaceae. But Harborne *et al.* (1971), proposed to divided the family Leguminosae into three sub-families, and his suggestion is nowadays confirmed by Veitch (2007), thanks to phylogenetic studies based on the analysis of DNA. The three sub-families are Papilionoideae (13,800 species), Caesalpinioideae and Mimosoideae.
Some authors used for family Leguminosae denomination Fabaceae (Valíček *et al.*, 2002).

1.1.2 Morphology

Habitus of Leguminosae can be tree, shrub, liana and herb. The size varies significantly from small erect, creeping or climbing herbs to trees reaching several metres.
The leaves are usually alternate or compound. They can be trifoliate *(Trifolium pratense, Medicago sativa)*, paripinnate *(Arachis hypogaea)* or imparipinnate *(Cicer arietinum)*, rarely palmately compound *(Lupinus albus)*. The Mimosoideae and Caesalpinioideae can be binnate *(Acacia senegal)* (Valíček *et al.,* 2002). There is often a pair of stipules at the base of the leaf petiole which in some species are fused with the low portion of petiole. Leaflets can be sessile or have petiolules which can have stiples, usually small and very narrow, at their bases. Young seedlings have two cotyledons; the first true leaf to appear is usually simple, with one leaflet (Bogdan, 1977). The leaves' margin can be entire or serrate.

The flowers are irregular, two-sided symmetrical and have specialized structure, so called "butterfly shape" (Whyte *et al.*, 1953). They are seldom single, mostly arranged into inflorescences - racemes, spikes, heads. The flower consists of calyx, corolla, stamens and a pistil. The calyx has five sepals usually green. The corolla consists of five petals. The biggest and upper petal is called "banner". The two side petals, the "wings", surround the two lowermost petals. The two lowermost petals are fused together forming a boat-shape structure so-called "keel", where there are the stamens. Corolla petals are variously coloured (white, yellow, pink, purple) and very bright to attract the pollinators, because Leguminosae are entomophilous (Bogdan, 1977).

Fruit is a dry dehiscent fruit, which opens along a seam on two sides. Legume develops from a simple carpel. It is one-capsule and can be one-seeded or many-seeded. Pericarp can be dry, skinny to hard. A common name for this type of fruit is a "pod" (Hnilička *et al.*, 2005).

Legumimosae frequently have a well-developed central tap-root, which carries numerous fine branches (Whyte *et al.*, 1953). The central tap-root can penetrate deep into the soil and in some cases side roots can be thickened and serve as a storage organ, that is why plants of this family can be easily tolerate to unpleasant conditions. On the fine branches there are root nodules where there are the symbiotic *Rhizobium* bacteria. The bacteria increase the nutritive value in case of fodder and enrich the soil with nitrogen so the agriculturists can save on nitrogen fertilizers (but in tropics has nitrogen fixation lesser extent, because it can occur only in moist soil) (Bogdan, 1977).

1.2 Origin and distribution

Leguminosae in general are of tropical origin. Most of plants are nowadays spread far from its indigenous occurrence. Species of Caesalpinioideae have remained almost exclusively tropical species represented mainly by trees, woody lianes and shrubs and by a relatively few herbs. The Mimosoideae have also a number of primitive features and are distributed mainly in the tropics and subtropics although a number of species has penetrated into warm temperate areas. The Papilionoideae is the most advanced subfamily with a great morphological variability and adaptation and its species penetrated all parts of the world, reaching arctic areas and high mountains it the tropics. The great majority are small shrubs and herbs (mostly annuals), and trees are in minority (Bogdan, 1977). Black and white map of distribution of plants of family Leguminosae adopted from the website of Missouri Botanical Garden (Anonymous, 2008) is shown as Fig. 1.

Fig. 1: Map of distribution of plants of family Leguminosae

1.3 Uses of Leguminosae plants

The Leguminosae constitute one of human‘s most important groups of plants. In agriculture Leguminosae are used as cover crops, as green manures and in shifting cultivation (Skerman *et al.*,1988). They are used for human consumption and for animals as a fodder. Especially Caesalpinioideae and Mimosoideae are used as timber plants. Leguminosae also synthesise a wide range of natural products that is why they are used for dyes, tannins, gums, resins. Thanks to content of rotenone, they are used as insecticides. Some plants are used as medicinal plants. And last but not least Leguminosae are used as ornamental plants (mainly thanks to the “butterfly flower”) (Harborne *et al*., 1971).

Leguminosae are used in agriculture mostly because of they can fix the aerial nitrogen. Thanks to well-developed central tap-root they can use nutrients from deep lays of soil and they keep water in the soil. For this purposes are used in tropics and subtropics e.g. *Pueraria phaseoloides* or *Stizolobium deeringion* (Skerman *et al.*, 1988).

For direct human consumption are used plants mainly from Papilionoideae, e.g. *Cicer arietinum, Glycine max, Lens culinaris, Phaseolus* sp. or *Pisum sativum* (Harborne *et al.*, 1971). They are rich in high quality proteins, fats and fibre. But human can use the Leguminosae also as oil and flavour crops. For this purposes are used e.g. *Arachis hypogaea, Tamarindus indica, Voandzeia subterranea* or *Glycine max* (Harborne *et al.*, 1971).

As fodder plants for animals are used mostly *Trifolium* sp., *Medicago sativa*, *Vicia sativa* or *Lotus corniculatus* (Harborne *et al.*, 1971).

For timber are often used *Acacia melanoxylon, Dalbergia latifolia, Pterocarpus santalinus, Robinia pseudoacacia* etc. (Harborne *et al.*, 1971).

For other purposes are used *Indigofera tinctoria, Acacia catechu* (dye), *Acacia senegal, Astragalus gummifer* (gum), *Derris* sp., *Lonchocarpus* sp. (insecticides) (Harborne *et al,*. 1971) and *Galega officinalis, Glycyrrhiza glabra, Trigonella foenum-graecum* (medicinal plants) (Hnilička *et al.*, 2005).

The economic importance of the family is likely to increase as human pressure places greater demand on marginal land. Many Legume species are characteristic of open and disturbed places and are thus well adapted to grow under poor conditions (Maesen *et al.*, 1989).

1.4 Isoflavonoids

1.4.1 Introduction

Flavonoids are plant secondary metabolites, consisting of two large subclasses – flavonoids in narrow sense, and isoflavonoids (Tahara and Ibrahim, 1995). Isoflavonoids differ form others classes of flavonoid by their greater structural variation, the greater frequency of isoprenoid substitution and their presence in plants in the free form rather than in glycosidic combination. Isoflavones are isomeric with widely occurring flavones (e.g. genistein is derived biosynthetically by an aryl migration from the same chalcone precursor as that which given rise to the flavone apigenin) (Harborne, 1994; Harborne *et al.*, 1999). Black and white drawings of apigenin and genistein adopted from Harborne *et al.* (1971) is shown as Fig. 2 and Fig. 3.

Fig. 2: Chemical structure of apigenin

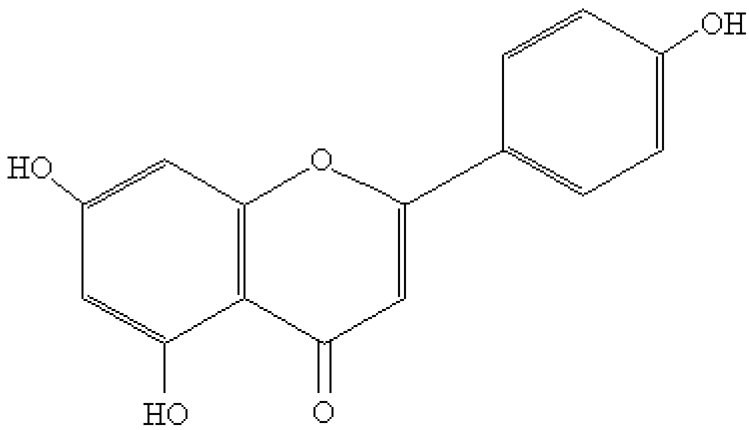
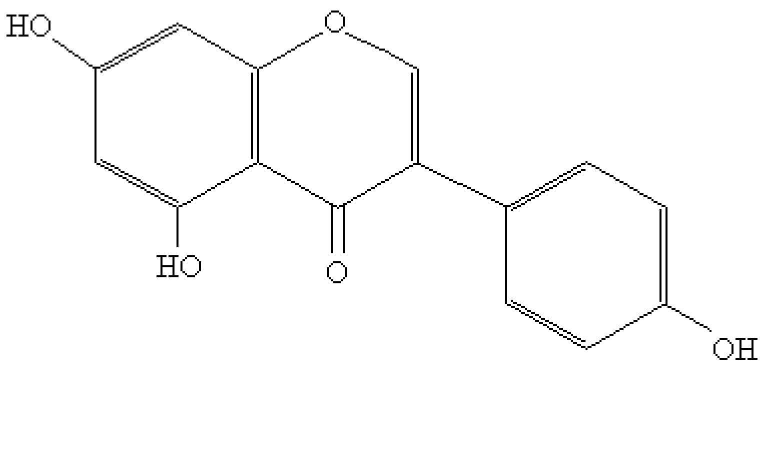


Fig. 3: Chemical structure of genistein



1.4.2 History of isoflavonoids

The discovery of isoflavonoids as natural products has its origin in the middle of nineteenth century, when Reinsch (1842) and Hlasiwetz (1855) obtained ononin from the root of *Ononis spinosa* L. Ononin was revealed after eighty years later as the 7-*O*-glucoside of formononetin (Veitch, 2007).

In 1984 it was known 465 structures, in 1989 it was published 630structures and in 1994 it was published 870 structures (Harborne, 1994). At the end 2004 was known as many as 1600 examples (Veitch, 2007).

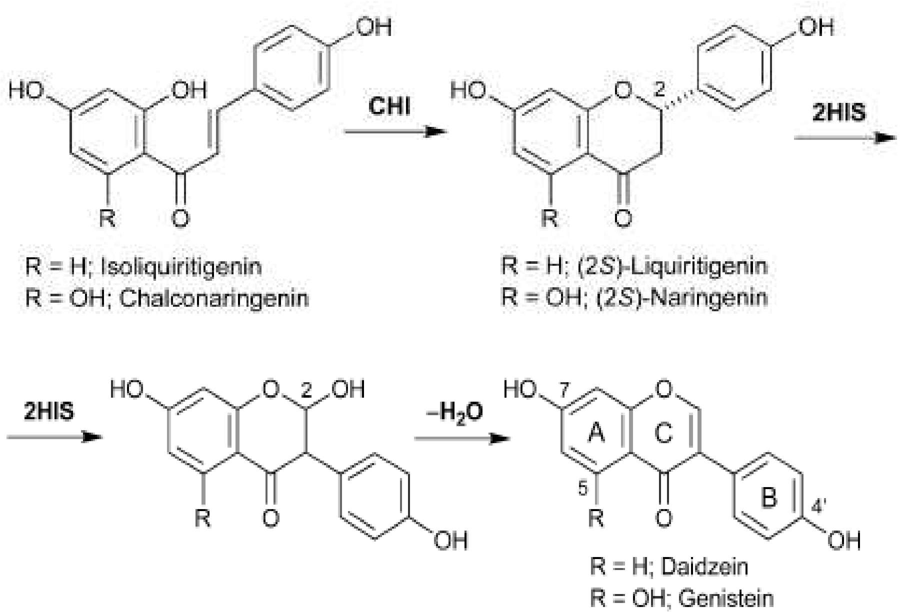
Discovery of new isoflavonoids is reflecting to the continued developing high level of research activity in this field (Harborne, 1994).

1.4.3 Synthesis of isoflavonoids

Isoflavonoids are isomers of flavonoids. Isomerism is formulated by the rearrangement of 2-phenylchronane to 3-phenylchromane (Bruneton, 1999).

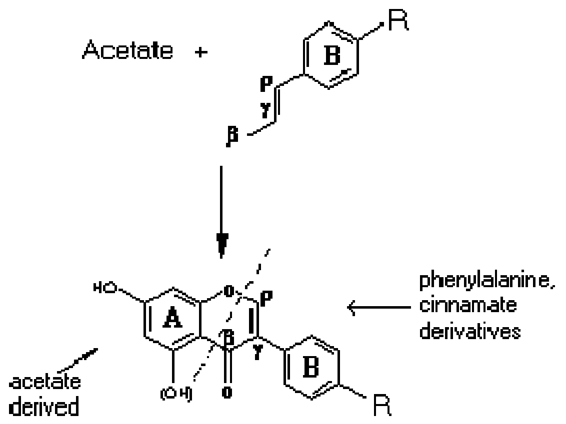
This rearrangement is generally depicted as a two-step process with two isoflavanones (liquiritigenin to daidzein) as intermediates in the following scheme on Fig. 4 adopted from Veitch (2007).

Fig. 4: Rearrangement process of isoflavonoids



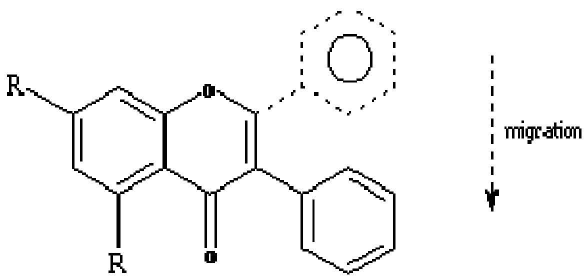
It has been established that acetate gives rise to ring A and that phenylalanine, cinnamate and cinnamate derivatives are incorporated into ring B and C-2, -3, and -4 of the heterocyclic ring on the following Fig. 5, adopted from the website of FaF, Veterinární a farmaceutické univerzity v Brně (Anonymous, 2006c).

Fig. 5: Rise of ring A and ring B



Since chalcones and flavanones are efficient precursors of isoflavonoids, the required aryl migration of ring B from the former 2 or beta position to the 3 or alpha position of the phenylpropanoid precursor must take place after formation of the basic C_{15} skeleton. This migration of ring B adopted from website FaF, Veterinární a farmaceutické univerzity v Brně (Anonymous, 2006c) is shown on Fig. 6.

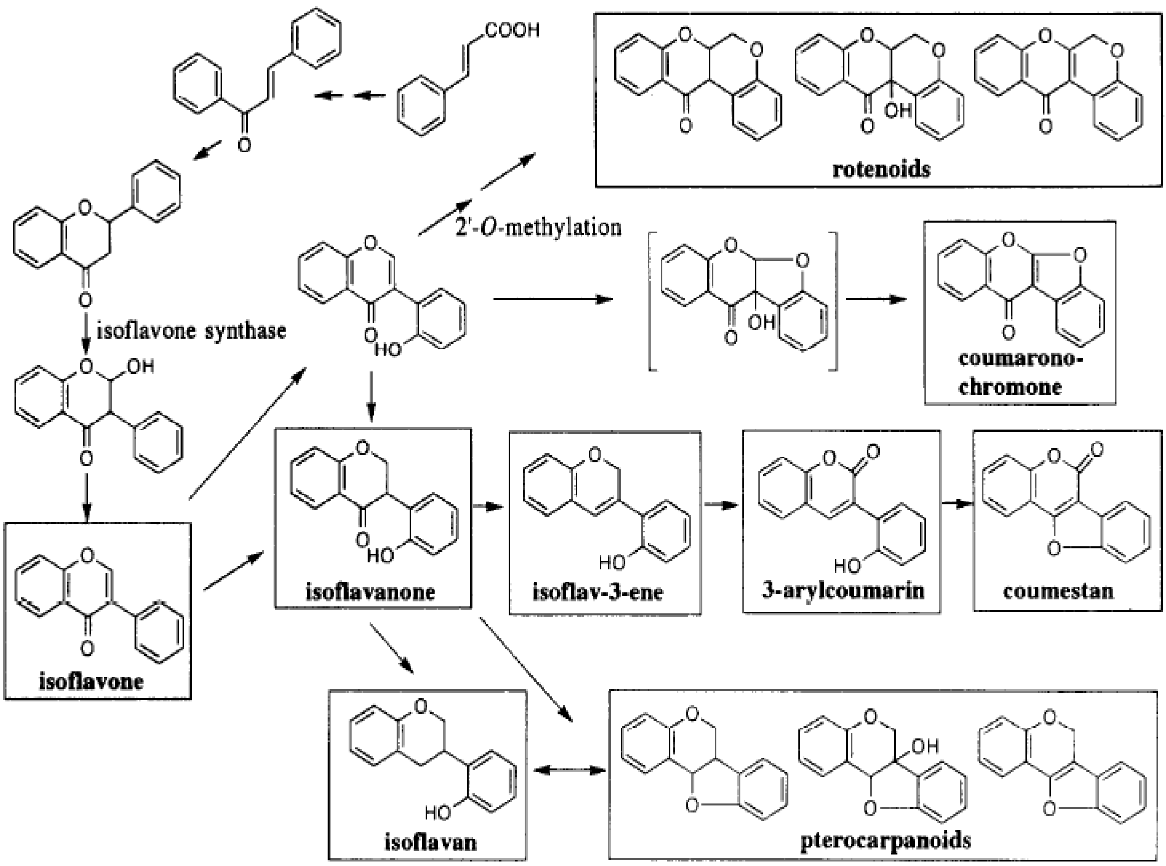
Fig. 6: Migration of ring B



1.4.4 Categorization of isoflavonoids

Isoflavonoids are divided into several subclasses to aid their systematic classification. According to the number of substituens on the basic 3-phenylchroman skeleton and mainly according to the different oxidation level of the central pyran ring (3-phenylchroman skeleton) isoflavonoids are divided into classes – isoflavones, isoflavanones, pterocarpans, isoflavans, isoflav-3-enes, 3-arylcoumarins, coumestans and rotenoids (Harborne, 1994; Tahara and Ibrahim, 1995; Harborne *et al.*, 1999). These major isoflavonoid classes and their biosynthetic relationships adopted from Tahara and Ibrahim (1995) are shown in Fig. 7.

Fig. 7: Major isoflavonoid classes



There are many different types of isoflavonoids, but all have got the same basic characteristic structure, and than, according to substituents $R_1 - R_6$, as shown in Table 2 [adopted from Klejdus *et al.* (2001)]. Black and white drawing of basic chemical structure of isoflavonoid adopted from Klejdus *et al.* (2001) is shown as Fig. 8.

Fig. 8 Basic chemical structure of isoflavonoid

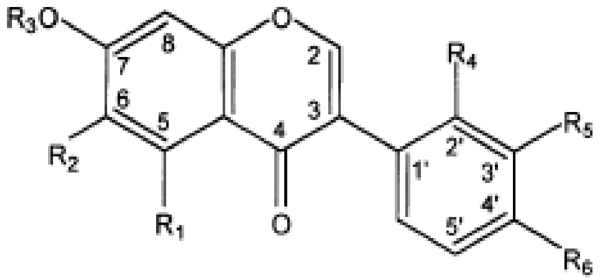


Table 2: Structures of isoflavones

Name of isoflavone	R_1	R_2	R_3	R_4	R_5	R_6
daidzin	H	H	glc	H	H	OH
glycetin-7-O-β-D-glucoside	H	OCH_3	glc	H	H	OH
calycosin-7-O-β-D-glucoside	H	H	glc	H	OH	OCH_3
genistin	OH	H	glc	H	H	OH
daidzien-7-O-β-D-glucoside-6"-O-malonate	H	H	glc-Mal	H	H	OH
3-methylorobol-7-O-β-D-glucoside	OH	H	glc	H	OCH_3	OH
pratensein-7-O-β-D-glucoside	OH	H	glc	H	OH	OCH_3
calycosin-7-O-β-D-glucoside-6"-O-malonate	H	H	glc-Mal	H	OH	OCH_3
pseudobaptigenin-7-O-β-D-glucoside	H	H	glc	H	O-	OCH_{2-}
dadzein-7-O-β-D-glucoside-6"-O-acetate	H	H	glc-OAc	H	H	OH
ononin (formononetin-7-O-β-D-glucoside)	H	H	glc	H	H	OCH_3
genistein-7-O-β-D-glucoside-6"-O-malonate	OH	H	glc-Mal	H	H	OH
orobol-7-O-β-D-glucoside-6"-O-malonate	OH	H	glc	H	OH	OH
3-methylorobol-7-O-β-D-glucoside-6"-O-malonate	OH	H	glc-Mal	H	OCH_3	OH
pratensein-7-O-β-D-glucoside-6"-O-malonate	OH	H	glc-Mal	H	OH	OCH_3
daidzein	H	H	H	H	H	OH
irilone-4'-O-β-D-glucoside	OH	O-	CH_{2-}	H	H	glc
pseudobaptigenin-7-O-β-D-glucoside-6"-O-malonate	H	H	glc-Mal	H	O-	OCH_{2-}
glycitein	H	OCH_3	H	H	H	OH

Table 2 (Continued)

Name of isoflavone	R_1	R_2	R_3	R_4	R_5	R_6
orobol	OH	H	H	H	OH	OH
calycosin	H	H	H	H	OH	OCH_3
formononetin-7-O-β-D-glucoside-6''-O-malonate	H	H	glc-Mal	H	H	OCH_3
afrormosin-7-O-β-D-glucoside	H	OCH_3	glc	H	H	OCH_3
sissotrin (biochanin A-7-O-β-D-glucoside)	OH	H	glc	H	H	OCH_3
irilin B-7-O-β-D-glucoside	OH	OCH_3	glc	OH	H	H
irilone-4'-O-β-D-glucoside-6''-O-malonate	OH	O-	CH_{2-}	H	H	glc-Mal
trifoside (prunetin-4'-O-β-D-glucoside)	OH	H	CH_3	H	H	glc
afrormosin-7-O-β-D-glucoside-6''-O-malonate	H	OCH_3	glc-Mal	H	H	OCH_3
pseudobaptigenin-7-O-β-D-glucoside-6''-O-acetate	H	H	glc-OAc	H	O-	OCH_{2-}
formononetin-7-O-β-D-glucoside-6''-O-acetate	H	H	glc-OAc	H	H	OCH_3
texasin-7-O-β-D-glucoside-6''-O-malonate	H	OH	glc-Mal	H	H	OCH_3
irilin B-7-O-β-D-glucoside-6''-O-malonate	OH	OCH_3	glc-Mal	OH	H	H
3'-methylorobol	OH	H	H	H	OCH_3	OH
genistein	OH	H	H	H	H	OH
biochanin A-7-O-β-D-glucoside-6''-O-malonate	OH	H	glc-Mal	H	H	OCH_3
pratensein	OH	H	OH	H	OH	OCH_3
prunetin-4'-O-β-D-glucoside-6''-O-malonate (trifoside malonate)	OH	H	CH_3	H	H	glc-Mal
pseudobaptigenin	H	H	H	H	O-	OCH_{2-}
irilone-4'-O-β-D-glucoside-6''-O-acetate	OH	O-	CH_{2-}	H	H	glc-OAc
formononetin	H	H	H	H	H	OCH_3
prunetin-4'-O-β-D-glucoside-6''-O-acetate (trifoside acetate)	OH	H	CH_3	H	H	glc-OAc
texasin	H	OH	H	H	H	OCH_3
biochanin A-7-O-β-D-glucoside-6''-O-acetate	OH	H	glc-OAc	H	H	OCH_3
irilone	OH	O-	CH_{2-}	H	H	OH
prunetin	OH	H	CH_3	H	H	OH
biochanin A	OH	H	H	H	H	OCH_3

1.4.5 Taxonomical distribution

Isoflavonoids are found regularly in family Leguminosae. They have been recorded occasionally in other angiosperm families, in gymnosperm sources and in moss. From non-plant sources we can mention a marine coral (*Echinopora lamellosa)* (Harborne, 1994). Families and genera known to contain isoflavonoid-producing species are given in Table 3 [adopted from Harborne (1994)].

Table 3: Isoflavonoids in non-legume plants

Family	Genera	Number of reported isoflavonoids
DICOTYLEDONS		
Amaranthaceae	*Iresine*	1
Celastraceae	*Euonymus*	1
Chenopodiaceae	*Beta*	2
	Salicornia	2
	Salsola	3
	Spinacia	1
Compositae	*Eclipta*	2
	Mutisia	1
	Simsia	1
	Wedelia	2
	Wyethia	8
Cruciferae	*Brassica*	1
Euphorbiaceae	*Macaranga*	2
Moraceae	*Cudrania*	3
	Maclura	2
Myristicaceae	*Osteophleum*	2
	Virola	5
Nyctaginaceae	*Boerhaavia*	7
Ochnaceae	*Ochna*	2
Polygonaceae	*Polygonum*	1
Rosaceae	*Coloneaster*	1
	Prunus	2
Scrophulariaceae	*Sopubia*	2
	Verbascum	1
Stemonaceae	*Stemona*	3
Zingiberaceae	*Costus*	2
MONOCOTYLEDONS		
Gramineae	*Festuca*	1
Iridaceae	*Belamcanda*	7
	Iris	26
	Patersonia	2
Liliaceae	*Hemerocallis*	1
GYMNOSPERMS		
Cupressaceae	*Juniperus*	6
Podocarpaceae	*Podocarpus*	5
BRYOPHYTES		
Bryaceae	*Bryum*	4

It is possible that isoflavonoids are more widespread than these in this table, but the lack of a satisfactory method for detection of isoflavonoids means that the status quo of their natural occurrence is imperfect (Harborne, 1994; Harborne *et al.*, 1999).

In non-plant sources, isoflavonoids were detected e.g. in beer (Lapčík *et al.*, 1998).

1.4.6 Biological properties of isoflavonoids

Isoflavonoids belong to the group of phytoestrogens. Phytoestrogens are fenolic matters imitating a structure of natural mammalian estrogens and making weak estrogenic activity. Food is main source of isoflavonoids for human population.

The existence of phytoestrogens was depicture for the first time by Bennetsem in 1946 in connection with the infertility of sheep, which were grazing on pastures with large scale of *Trifolium subterraneum* for the long time. In 1954 Bradbury and White isolated from that genistein and formononetin, isoflavonoids with estrogenic effect. Infertility was established at sheep for the first time, because cattle are less sensitive to the effect of phytoestrogens (Anonymous, 2006c).

1.4.6.1 Importance of isoflavonoids for plants

Plants produce a lot of organic matters, which do not affect directly growing and progression, so called secondary metabolites. The primary metabolites are basic element for the biosynthetic production of secondary metabolites, but exact border between primary and secondary metabolites does not exist. Some of these metabolites are often exactly divided into several taxonomic groups inside the plant kingdom. A lot of their functions are still unknown.

Natural plant secondary metabolites can be divided into three main groups: terpenoids, alkaloids and phenylpropanoids with their relative phenolic compounds. Individual groups of phenolic matters have many conjunctive signs, which are outgoing from their biochemical pathways. One of the most significant groups of phenolic compounds are flavonoids. This gathering consists of many groups of plant metabolites, where chalcons, aurons, flavonons, isoflavonoids, flavonols, catechins and anthocyanidins belong to.

Isoflavonoids make significant subgroup of flavonoids. They are positional isomer of flavonoids, but they are less spread in plant kingdom than flavonoids and this is conspicuous that isoflavonoids are in less number of families.

Content of isoflavonoids in plant is affected by many biotic and abiotic factors. Isoflavonoids occur as constitution matters or they can appear by incidence of stress. Isoflavonoids play important role in some function in defensive system of a plant – natural barrier against infection during germination of seeds, attack by insect or damage by pests.

These compounds can sustain for certain time theirs biological activity and can influence microbiological proportions in soil or can be used by people – e.g. antifungal properties (Harborne *et al.*, 1999; Klejdus, 2004).

1.4.6.2 Importance of isoflavonoids for animals

The pharmacological properties of isoflavonoids are seldom known. The sole activity which has found an application is the insecticide activity of rotenoids. Estrogenic properties are also known: isoflavonoids cause infertility in sheep that ingest massive quantities of clover; cows seem less susceptible, perhaps because of a difference in metabolism (Bruneton, 1999).

In animal models, a soybean-based diet decreases mammary and prostrate carcinogenesis. Pure genistein is also an anticarcinogen (mammary tumors of the female rat) (Bruneton , 1999).

Leguminosae also products isoflavonoids, which kills animals – rotenoids used as insecticides or piscidals (rotenone inhibits the electron transport pathway in mytichondria and is therefore toxic to all forms of life) (Harborne *et al.*, 1999).

1.4.6.3 Importance of isoflavonoids for human

The occurrence of isoflavones in food raises the question of their potential impact on human health. In the soybean, the concentration of daidzein, genistein and their glycosyl derivates can reach 3 g / kg. The same compounds are also found in all of the derived products (soybean powder, milk, fermented products), at concentrations that vary depending on the industrial manufacturing process.

These isoflavones and their intestinal metabolites (equol, demethylangolensin) bind to estrogen receptors, and most often, they have a weak estrogenic activity. They are also tyrosine-kinase inhibitors which may have a role in the transformation and cell proliferation phenomena (Bruneton, 1999).

Epidemiologic studies in human strongly suggest the low incidence of hormonedependent diseases that is observed in Asian and vegetarian populations, correlated with the high consumption of soybean which is common in those cultures (analysis of phytoestrogens by gas chromatography-mass spectrometry). The soybean isoflavones, and maybe other constituents as well, seem to have a preventive effect on breast and prostate cancer, as well as colorectal cancer.

Several recent studies suggest that isoflavones and soybean decrease the symptoms of menopause (hot flashes and others) and reduce the risk of osteoporosis (Bruneton, 1999).

1.4.7 Methods of detection

1) Extraction methods of isoflavonoids

Extraction, means separation of material, is the first step of qualitative and quantitative analysis. It should be fast, simple, cheap and automatic. There are many possibilities of extraction – Solid-Liquid Extraction, enzymatic hydrolysis, Supercritical Fluid Extraction (SFE), Microwave-Assisted Extraction (MAE) or Accelerated Solvent Extraction (ASE) (Klejdus, 2004).

2) Cleaning and fraction of isoflavonoids

Heterogeneousness of composition of raw extract lead to other separation step – fraction, elimination and to graduate the specimen. It can be used Liquid-Liquid Extraction (LLE), Column Chromatography (CC), Thin Layer Chromatography (TLC), Paper Chromatography (PC) or Solid Phase Extraction (SPE) (Klejdus, 2004).

3) Methods of determination and identification

Detection and identification of biologically active compounds is the main status of the research. Route-identification can afford Paper Chromatography (PC), Thin Layer Chromatography (TLC) or Column Chromatography (CC). But for determination of individual isoflavonoids are used Gas Chromatography (GC), High-Performance Liquid Chromatography (HPLC), Capillary Electrophoresis (CE) and Enzyme-Linked Immuno Sorbent Assay (ELISA) (Klejdus, 2004).

4) Methods of detection

Isoflavonoids are detected in plants tissues by their phenolic reactions and the different subclass can be distinguished by their ultraviolet spectra (Harborne *et al.,* 1999). It is common to use HPLC in combination with UV detection, but more efficient is the combination of HPLC-MS (mass spectrometry) or HPLC-APCI (atmospheric pressure chemical ionization) (Boland and Donely, 1998). Capillary electrophoresis (CE) in the determination of isoflavonoids is also used, in comparison with HPLC, the CE method is rapid and does not consume solvent. CE in combination with ESI-MS (electrospray inoization-mass spectrometry) has been shown to be better for determination of isoflavonoids than CE alone (Boland and Donely, 1998).

Structural elucidation of natural products is facilitated by the rapid progress in nuclear magnetic resonance (NMR) spectroscopic techniques (Harborne, 1994) and nowadays it is routine method for structure elucidation of isoflavonoids (Boland and Donely, 1998).

2 Objectives

The aim of this study is identification (by literature data analysis) of plant species belonging to the family Leguminosae (with special focus on underutilised pulses or legumes) of tropical and subtropical origin (especially native in Asia, Africa and America) as prospective sources of biologically effective isoflavonoids. The list of the species and isoflavonoids, which seem to be the most prospective for further phytochemical analysis, is provided as a main result of this work. In addition, botany, origin and distribution and uses of Leguminosae plants and chemistry of isoflavonoids of family Leguminosae are summarised.

3 Materials and Methods

At the beginning of the work is necessary to emphasize, that the family Leguminosae is the 3rd largest family in the entire world, that's why the definite draft has to be made. Selection of tropical and subtropical useful plants was accomplished according to the publication focused on this type of plants in South-East Asia (Maesen and Somaatmadja, 1989), and according to the publication focused on species incident and used as pulses mainly in area of Africa (Grubben and Danton, 2004).

For the selection of tropical Leguminosae of the New world the internet database ILDIS (= International Legume Database & Information Service) was used. The plants were selected according to key words “Uses” and “Geographical records”. The last selection was accomplished with help of Amazonian Ethnobotanical Dictionary (Duke and Martinez, 1994) and other books dealing with tropical useful plants (Rehm and Espig, 1991; Valíček *et al.*, 2002).

All the proceedings were consulted with my thesis supervisor and together we made a decision that this is the best way of selection of materials.

The following part was verification of the authors who had described the plant as the first and synonyms for each plant from the list. This was carried out with the help of internet database IPNI (=The International Plant Names Index). For the main purpose of this thesis it was used database IK (Index Kewensis).

If some plant did not have an author, it was used Mancfeld´s book to decide (and such plants are marked with “*” in the list).

All data were gathered until 29th February 2008.

The final stage of the work consisted in the most detail scan of all names and its synonyms at standpoint of content of isoflavonoids. During working upon this target it was progressed in a following way – in database WOS (= Web of Science) were ordered as a “key word” 1) the name of the plant, and for literary reiews 2) “isoflav* AND legum*”. Than were chosen from the articles substantial information – which plant contains isoflavonoids, which type of isoflavonoids and from which part of plant the isoflavonoid were isolated. Then the data were processed into a detailed table (see Table 4) and were suggested the found-out results at the discussion at the end of the thesis to further research.

4 Results

The results of this work are shown in Table 4. It contains the scientific name of the plant according to IPNI, synonyms, the part of the plant, from which were neat isoflavonoids isolated, names of the main contained isoflavonoids and origin and main geographical areas of distribution of the plant. References are included at the end of the table.

As a result it was found, that 45 plants *(Abrus precatorius, Arachis hypogaea, Baphia nitida, Cajanus cajan, Ceratonia siliqua, Cicer arietinum, Crotalaria pallida, Cytisus scoparius, Dalbergia monetaria, Dipteryx odorata, Erythrina berteroana, Erythrina variegata, Galega officinalis, Gliricidia sepium, Glycine max, Indigofera linnaei, Lathyrus sativus, Lens culinaris, Lotus corniculatus, Lupinus albus, Lupinus mutabilis, Macrotyloma geocarpum, Macrotyloma uniflorum, Medicago sativa, Phaseolus coccineus, Phaseolus lunatus, Phaseolus vulgaris, Pisum sativum, Pithecellobium dulce, Pongamia pinnata, Psophocarpus tetragonolobus, Pterocarpus marsupium, Sesbania grandiflora, Sesbania sesban, Tephrosia purpurea, Trifolium pratense, Trifolium repens, Trigonella foenum-graecum, Vicia faba, Vigna adenantha, Vigna angularis, Vigna mungo, Vigna radiata, Vigna subterranea and Vigna unguiculata)* of 170 chosen tropical and subtropical useful plants of family Leguminosae contain isoflavonoids, 2 of chosen plants contain homoisoflavonoids *(Caesalpinia digyna, Caesalpinia pulcherrima)* and 1 plant contains isoflavanquinone *(Abrus precatorius).*

Interestingly, 125 of remaining plants used locally in various tropical or subtropical regions as leguminous plants, namely *Acacia leucophloea, A. macracantha, A. senegal, Adenanthera pavonina, Afzelia xylocarpa, Albizia acle, A. altissima, Alysicarpus rugosus, Amblygonocarpus andongensis, Amburana cearensis, Bauhinia petersiana, B. racemosa, B. vahlii, B. variegata, Calpocalyx brevibracteatus, Campsiandra angustifolia, Canavalia ensiformis, C. gladiata, C. maritima, C. rosea, Cassia bicapsularis, C. fistula, C. floribunda, C. tora, Castanospermum australe, Cercis siliquastrum, Clitoria ternatea, Copaifera paupera, C. reticulata, Cordeauxia edulis, Craibia brownii, Crotalaria juncea, C. karagwensis, C. lachnophora, C. retusa, C. vitellina, Cyamopsis tetragonoloba, Desmodium repandum, Dialium cochinchinense, Dipteryx micrantha, D. polyphylla, Entada phaseloides, E. pursaetha, Erythrina edulis, Faidherbia albida, Chamaecrista absus, Indigofera cordifolia, I. glandulosa, I. linifolia, Inga cinnamomea, I. edulis, I.*

laurina, I. macrophylla, Inocarpus fagiferus, Intsia bijuga, Lablab purpureus, Lathyrus japonicus, L. odorathus, Leucaena leucocephala, L. pulverulenta, Lotus glaber, Lysidice rhodostegia, Macroptilium lathyroides. Macrotyloma uniflorum, Machaerium lunatum, Medicago polymorpha, Melilotus officinalis, Mucuna gigantea, M. poggei, M. pruriens, M. sloanei, Ormosia coccinea, O. paraensis, Padbruggea dasyphylla, Pachyrhizus ahipa, P. ferrugineus, P. tuberosus, Parkia biglobosa, P. intermedia, P. leiophylla, P. multijuga, P. nitida, P. roxburi, P. speciosa, Parkinsonia aculeata, Pentaclethra macroloba, Phaseolus acutifolius, Pithecellobium bubalinum, P. fagifolium, P. jiringa, P. unguis-cati, Prosopis flexuosa, P. hassleri, P. chilensis, Psophocarpus scandens, Pterocarpus santalinoides, Ramorinoa girolae, Samanea saman, Saraca dives, Senna hirsuta, S. obtusifolia, S. occidentalis, S. sulfurea, Sesbania bispinosa, Shuteria hirsuta, Tamarindus indica, Teramnus labialis, Tylosema esculentum, Vatovaea pseudolablab, Vicia hirsuta, V. sativa, Vigna aconitifolia, V. dalzelliana, V. marina, V. minima, V. pilosa, V. sublobata, V. trilobata, V. umbellata, V. vexillata, Xeroderris stuhlmannii, Xylia evansii, and *X. xylocarpa* have not been systematically analyse for qualitative and quantitative content of isoflavonoids. Moreover, some of these plants are commonly use legumes, which are grown all over the world as important economic crop, e.g. *Tamarindus indica*, *Lablab purpureus* or *Phaseolus acutifolius*.

5 Discussion

According to my review it was realised, that the most extended isoflavonoids in tropical and subtropical Leguminosae are genistein, daidzein, medicarpin and maybe we can attached here also biochanin A. This information is correspondence with review and articles of other authors, e.g. Veitch (2007). The results of my work indicate that genistein, daidzein and medicarpin were previously detected in 14, 10 and 7 plants, respectively.

Both our and other authors' results show that daidzein and genistein are the major types of isoflavonoids occurring in the tropical and subtropical plants of family Leguminosae. But there are also some minor types of isoflavonoids, which were discovered only in one plant and they have only the systematic scientific name, they have not the trivial name yet.

In general, our results on content of the main isoflavonoids in Legumes such as genistein, daidzein, medicarpin and biochanin A are in correspondence with previously published review articles (Zallocchi and Pomilio, 1994; Boland and Donnelly, 1998; Mazur *et al.*, 1998; Veitch, 2007). However, during the analysis of literature we have identified that among all 45 plants discovered to contain isoflavonoids 7 of them contain also coumestrol as one of the most abundant isoflavonoids.

It is also important to explore if plants containing isoflavonoid-like compounds e.g. homoisoflavonoids *(Caesalpinia digyna, Caesalpinia pulcherrima)* or isoflavanquinone *(Abrus precatorius)* also contain common types of isoflavonoids.

Soya *(Glycine max)* is nowadays the most physiologically active plant; it has got isoflavonoids in all parts of plant and in large scale. But future research can show us, that soya is not the only one.

6 Conclusion

In summary, it was found that 45 plants of 170 chosen tropical and subtropical useful plants of family Leguminosae contain isoflavonoids. Among them, several plants frequently used in human or animal nutrition, e.g. *Cicer arietinum* (Fig. 9), *Glycine max* (Fig. 10), *Arachis hypogaea* (Fig. 11), *Lens culinaris, Medicago sativa, Phaseolus coccineus* (Fig. 12) or *Vicia faba* have been identified.

The main isoflavonoids in the food crops are genistein, daidzein, medicarpin and biochanin A. But according to this review we can include among these most extended isoflavonoids also coumestrol, which was noticed in 7 plants and which is not enlarged as a main (respectively most extended) isoflavonoid.

Even though that scientists have been engaged in the investigation of isoflavonoids for quite a long time, the results of this investigation show that there is still 125 remaining plants, which have not been analysed for presence of isoflavonoids. Interestingly, among these 125 not-analysed plants, a few species with quite large economic importance such as *Tamarindus indica* (Fig. 13, Fig. 14) *Lablab purpureus* or *Phaseolus acutifolius* have been identified in this study.

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Appendices

Fig. 9: *Cicer arietinum* L. (dried seeds) (original photo by Anonymous, 2006d)

Fig. 10: *Glycine max* Merr. (fresh pods with seeds) (original photo by Anonymous, 2006e)

Fig. 11: *Arachis hypogaea* L. (pods with seeds) (original photo by Anonymous, 2007b)

Fig. 12: *Phaseolus coccineus* L. (seeds) (original photo by Anonymous, unknown)

Fig. 13: *Tamarindus indica* L. (pods) (orignal photo by Anonymous,2006f)

Fig. 14: *Tamarindus indica* L. (fruit pulp) (original photo by Anonymous, 2006g)