Doctoral Dissertation Abstracts...

"EVALUATION OF AMPHIPHILIC β-CYCLODEXTRINS AS NOVEL EXCIPIENTS IN THE PREPARATION OF NANOPARTICULATE DRUG DELIVERY SYSTEMS"

EREM MEMİŞOĞLU
Co-tutele Ph. D. Thesis
Hacettepe University (Turkey)-Université de Paris-Sud (France)
Pharmaceutical Technology Programme
Supervisors: Prof. Dr. A. Atilla Hıncal (Hacettepe University, Faculty of Pharmacy, Department of Pharmaceutical Technology, Ankara, Turkey) and Prof. Dr. Dominique Duchene (Université de Paris-Sud, Faculté de Pharmacie, Laboratoire de Physicochimie, Biopharmacie et Pharmacotechnie, UMR CNRS 8612, Chatenay-Malabry, France)
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In this study, amphiphilic β-cyclodextrins modified on the primary or secondary faces were synthesized, chemically characterized by ^{1}H NMR, FAB MS, DSC, FT IR spectroscopy and elemental analysis to ensure their purity and structural integrity. Their amphiphilic properties and behaviour in oil/water and air/water systems were demonstrated by surface pression and surface tension experiments. Inclusion forming capacities of these CD derivatives were also evaluated to demonstrate the formation of inclusion complexes by the co-lyophilization technique. Amphiphilic β-cyclodextrins; modified by acyl chains of different length (C6 and C14) and structures (linear and branched) on different faces (primary and secondary) with different bonds (ester or amide) yielded nanocapsules and nanospheres by the nanoprecipitation technique without using surfactants. Nanocapsule size and time-dependent physical stability are influenced by the structure and concentration of amphiphilic β-cyclodextrin.

Inclusion forming capabilities of amphiphilic β-cyclodextrins were demonstrated with three model drugs; bifonazole, clotrimazole and progesterone. Nanospheres prepared from these pre-formed inclusion complexes exhibited significantly higher loading and considerable reduction of burst effect. Hemolytic activity of amphiphilic β-cyclodextrins are significantly lower than natural β-cyclodextrin. Moreover, minimum inhibitory concentration of bifonazole and clotrimazole against *Candida albicans* have been significantly reduced by association to amphiphilic β-cyclodextrin nanospheres.

KEYWORDS: Amphiphilic β-cyclodextrins, characterization, inclusion complex, nanocapsule, nanosphere, loading, release, bifonazole, clotrimazole, progesterone

PHYTOCHEMICAL AND BIOLOGICAL STUDIES ON SOME TURKISH MEDICINAL PLANTS

Şebnem Harput
Supervisor: Prof. Dr. Y. Ogihara (Nagoya City University, Faculty of Pharmaceutical Sciences, Nagoya-Japan),
Prof. Dr. I. Saracoglu (Hacettepe University, Faculty of Pharmacy, Ankara, Turkey),
Date of examination: March, 2002.

The use of herbal medicine is a traditional way to treat illness and herbal medicine has been applied for more than millennia in several civilizations. The major challenge today is the discovery of plants with promising activities and the isolation of active principles. In our continuous research on Turkish medicinal plants, we focused on Scrophulariaceae and Lamiaceae families. In this dissertation, chemical components of *Veronica* species and *Phlomis* species have been evaluated together with their biological activities since they are widely distributed in Turkish flora and used as a folk medicine in Turkey.

Anti-inflammatory and cytotoxic activities of five *Veronica* species, *V. pectinata* var. *glandulosa*, *V. persica*, *V. hederifolia*, *V. polita* and *V. cymbalaria* were evaluated. Their methanol extracts showed both the inhibitory activity of nitric oxide (NO) production in lipopolysaccharide-stimulated macrophages and cytotoxic activity against KB and B16 cells. Methanol extracts were fractionated between water and chloroform. While water fractions significantly inhibited NO production without any cytotoxicity explaining the usage of these plants for the inflammatory diseases, chloroform fractions showed cytotoxicity dose-dependently. Concerning the bioactivity results, phytochemical studies were performed on the water fractions of *V. pectinata* var. *glandulosa*, *V. persica* and *V. hederifolia*. The use of chromatographical techniques and extensive 1D and 2D NMR spectroscopy led to the isolation and structure determination of 26 compounds including eleven iridoid glucosides, eight phenylethanoid glycosides and four flavonoid glycosides as well as one cyanoglucoside, one megastigman glucoside and one hexitol. Seven of them were isolated from the nature for the first time in this study.

In a continuation of the systematic studies on Turkish *Phlomis* species, antioxidant activity of *P. pungens* var. *pungens* was tested. The aqueous extract, phenylethanoid and iridoid fraction, major components of the phenylethanoid fraction exhibited protective effect against free radical-induced impairment of endothelium dependent relaxation in isolated rat aorta. In addition, phytochemical studies on *P. pungens* var. *pungens*, *P. lycia*, *P. sieheana*, *P. grandiflora* var. *fimbrilligera* and *P. fruticosa* were resulted to the isolation of 18 compounds including four iridoid glucosides, ten phenylethanoid glycosides one of which was new, two lignan glycosides together with one caffeic acid ester and one monoterpene glucoside.

Key words: *Veronica* species, anti-inflammatory activity, cytotoxic activity, free radical scavengers, *Phlomis species*, phenylethanoid glycoside, iridoid glycoside and flavonoid glycoside.

INVESTIGATION OF SOME PLANTS GROWING IN TURKEY FRO THA VIEW POINT OF ACETYLCHOLINESTERASE INHIBITORY ACTIVITY

İlkay ORHAN
Supervisor: Prof. Dr. Bilge ŞENER
Date of Examination: June, 28, 2002

In this study, it was aimed to evaluate the acetylcholinesterase inhibitory activity of some plants growing in Turkey.

Acetylcholinesterase inhibitors are the compounds which play role in the treatment of Alzheimer's Disease as well as myasthenia gravis, glaucoma and helminthiazis by preventing reduction of acetylcholine amount via inhibiting acetylcholinesterase enzyme that hydrolyze the neuromediator called acetylcholine in the neuronal end from which it is released and in the mechanism of action of insecticide drugs.

According to the literature survey, the plant species, on which no research have been done previously, from *Lycopodiaceae (L. annotinum, L. alpinum, L. clavatum, L. complanatum subsp. chamaesiparissus and L. selago),* Amaryllidaceae *(Galanthus elwesii, G. ikariae, Narcissus tazetta* subsp. *tazetta, Leucojum aestivum* and *Pancratium maritimum),* Fumarioideae (*Fumaria asepala, F. capreolata, F. cilicica, F. densiflora, F. judaica, F. kralikii, F. macrocarpa, F. parviflora, F. petteri* subsp. *thuretii, F. vaillantii* and *Corydalis solida* subsp. *solida*) and Buxaceae (*Buxus sempervirens*) families as well as Zygophyllaceae family (*Tribulus terrestris* and *Zygophyllum fabago*) considering their use in the folk medicine with Fabaceae (*Vicia faba*), were investigated from the view point of their acetylcholinesterase activity. Activity determinations were carried out by Ellman method which is a spectrophotometric, in vitro robotic screening method. Bioactivity-directed fractionation and isolation studies were carried on *Lycopodium clavatum, Galanthus ikariae, Narcissus tazetta* subsp. *tazetta* and *Fumaria vaillantii* which showed 50 % and more inhibitory activity in the prescreening program.

α-Onocerin (L-1), a triterpenoid isolated by preparative TLC from *Lycopodium clavatum,* was found to be the responsible compound for the activity.

In total, six Amaryllidaceae type alkaloids called lycorine (G-1), tazettine (G-2), crinine (G-3), galanthamine (G-4), 3-epi-hydroxybulbispermine (G-5) and 2-demethoxymontanine (G-6) from the active fractions of *Galanthus ikariae* were obtained by column chromatography.

In addition, lycorine (N-1), tazettine (N-2), N-norgalanthamine (N-3), haemantamin (N-4) and 3-epi-hydroxybulbispermine (N-5) were isolated from the active fractions of *Narcissus tazetta* subsp. *tazetta* as the Amaryllidaceae alkaloids.

The isoquinoline alkaloids called canadine (F-1), hydrastine (F-2), ophiocarpine (F-3), bulbocapnine (F-4), fumarophycine (F-5), corydaldine (F-6), ophiocarpine-N-oxide (F-7), protopine (F-8), β-allocryptopine (F-9) and berberine (F-10) were obtained from the active fractions of *Fumaria vaillantii.*

Although *G. ikariae* and *N. tazetta* subsp. *tazetta* extracts showed 75.56 % and 46.62 % inhibition, respectively; it made us considered that the activity of the extracts lower than 50 % resulted from the synergistic interaction between the alkaloids isolated. It was established that the compounds responsible for the inhibitor activity of *F. vaillantii* extract (94.23 %) were ophiocarpine, ophiocarpine-N-oxide, protopine, β-allocryptopine and berberine and there was also synergism in the interaction between the alkaloids.

PHARMACOGNOSTICAL STUDIES ON GLOBULARIA SPECIES

Hasan KIRMIZIBEKMEZ
Supervisor: Prof. Dr. İhsan ÇALIŞ, Hacettepe University, Faculty of Pharmacy, Dept. of Pharmacognosy, 06100, Sıhhiye, Ankara, Turkey.
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In this study, *G. trichosantha, G. orientalis, G. davisiana, G. cordifolia* and *G. dumulosa* (Globulariaceae) were investigated from the point of view of the isolation of their secondary metabolites, structure elucidation and radical scavenging (antioxidant) properties. The plant materials used in this study were extracted with either methanol or ethanol. After evaporation, the extracts were dissolved in water and extracted with chloroform in order to get rid of the lipophilic contents. The water-soluble parts of the crude extracts were used for isolation studies. Prefractionation and purification studies on the extracts were performed by using chromatographic methods (VLC, OCC, and MPLC). Consequently, 11 compounds, two of which were new, from the underground parts of *G. trichosantha;* 12 compounds from the aerial parts as well as 6 compounds, one of which was new, from the underground parts of *G. orientalis;* 17 compounds, one of which was new, from the aerial parts of *G. davisiana;* 19 compounds, three of which were new, from the underground parts of *G. cordifolia;* and 15 compounds, two of which were new, from the aerial parts of *G. dumulosa* were obtained. The structures of the compounds were elucidated by spectroscopic (UV, IR, 1D and 2D NMR and MS) and chemical (partial methylation, alkaline hydrolysis, reduction and acetylation) methods. Totally, 80 compounds, corresponded to 49 different structures were isolated and categorized under 7 main groups: Iridoids, phenylethanoid glycosides, sugar esters, lignan glycosides, flavon glycosides, acetophenone glycoside and sterols. The free radical scavenging (antioxidant) properties of the extracts and obtained phenolic compounds were determined by reduction of DPPH radical method.

Keywords: *Globularia trichosantha, Globularia orientalis, Globularia davisiana, Globularia cordifolia, Globularia dumulosa,* Globulariaceae, iridoids, phenylethanoid glycosides, sugar esters, lignan glycosides, flavon glycosides, acetophenone glycoside, sterols, free radical scavenging property.

Doctoral Dissertation Abstracts...

PHARMACOGNOSTICAL RESEARCHES ON SOME PHLOMIS SPECIES

Funda Nuray Yalçın

Supervisor : Prof. Dr. Tayfun Ersöz, Department of Pharmacognosy, Faculty of Pharmacy, Hacettepe University, 06100, Ankara, Turkey.

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The genus *Phlomis* is represented by 34 species in Turkish flora. The genus is classified into three groups (Group A, Group B and Group C) In this study, three *Phlomis* species, each belongs to a different group have been searched from the point of view of their chemical constituents by means of phytochemical investigations on their overground parts.

The overground parts of the plants were extracted with methanol. The crude extract was dissolved in water and extracted with hexane, chloroform and n-BuOH, succesively. The same extraction method has been applied for three species. By means of a serial chromatographic studies on the n-BuOH extracts, shanzhiside methylester, martynoside, 4''-O-acetyl martynoside, samioside, 2,6-dimethoxy-4-hydroxyphenol-1-O-β-D-glucopyranoside, uridine and phlomuroside, 1-methyl-β-D-gluco pyranoside from *Phlomis samia*; lamiide, ipolamiide, verbascoside, forsythoside B, alyssonoside and syringaresinol-4'-O-β-D-glucopyranoside, from *P. monocephala*, and verbascoside, syringin, dihydrosyringin, coniferin, 2,6-dimethoxy-4-hydroxyphenol-1-O-β-D-glucopyranoside picein, betulalbuside A, 8-hydroxylinaloyl-3-O-β-D-glucopyranoside and 1-methyl-β-D-glucopyranoside from *P. carica* were isolated.

Radical scavenging activity of the water and n-BuOH extracts prepared from the plant specimens and the compounds isolated were tested towards the 2,2,-diphenyl-1-pirylhydrazyl (DPPH) radical in TLC system.

Key Words: *Phlomis samia, Phlomis monocephala, Phlomis carica,* Lamiaceae, iridoid glucoside, phenylethanoid glycoside, lignan glucoside, phenolic glucoside, acetophenone glucoside, monoterpene glucoside, megastigmane glucoside.

PHYTOCHEMICAL AND BIOLOGICAL INVESTIGATIONS ON A TURKISH AJUGA SPECIES, AJUGA SALICIFOLIA

Pınar AKBAY

Supervisor : Prof. Dr. Otto Sticher*, Coexaminers: Prof. Dr. Ihsan Çalış**, Prof. Dr. August Schubiger*, Dr. Jörg Heilmann*, * ETH-Zürich (Swiss Federal Institute of Technology), Dept. of Applied Biosciences, Inst. of Pharmaceutical Sciences, Winterthurerstr. 190, 8057 Zurich, Switzerland. **Hacettepe University, Faculty of Pharmacy, Dept. of Pharmacognosy, 06100, Sıhhıye, Ankara, Turkey.

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In the flora of Turkey, the genus *Ajuga* L., commonly named as bugle, is represented by 11 species some of which are traditionally used in wound healing, as diuretic, as well as against diarrhea and high fever.

Ajuga *salicifolia* was collected near Ankara-Beytepe in July 1998. Air-dried and powdered aerial parts (1 kg) were extracted successively with PE, DCM, EtOAc, MeOH, and MeOH-H_2O (1:1). After a TLC control, the DCM and EtOAc extracts were combined. The fractionation of the DCM/EtOAc and MeOH extracts by means of various chromatographic methods (VLC, CC, MPLC and HPLC) led to the isolation of 18 compounds, 10 of which were new to the literature. Structures of the isolates were established by extensive use of 1D- and 2D-NMR (COSY, HSQC, HSQC-TOCSY, HMBC) experiments. Additional information was gathered by mass spectrometry (FAB-, ESI- and HRMAL-DIMS) UV spectroscopy, physical ($[\alpha]_D$ and chemical (acid hydrolysis) methods. The relative stereochemistry was determined from the results of 2D ROESY or NOESY experiments.

The majority (nine) of the isolated compounds were new stigmastane-type sterols, showing a high structure variability. Two of them were novel sterol glycosides, displaying three epoxidations on the stigmastan-7-en skeleton, which enabled the presence of three additional (five, six and eight membered) ring systems. The other secondary metabolites were ionone, iridoid and phenylethanoid glycosides. The ionone glycosides were reported for the first time from *Ajuga* species. One of them was new to the literature. Except the ubiquitous compounds harpagide and 8-*O*-acetylharpagide, the isolated iridoid and phenylethanoids were new for *A. salicifolia*. Furthermore, this is the first report of 8-*O*-acetylmioporoside from the family, Lamiaceae.

All the isolated compounds were tested in in-house assays. Some of the sterols showed significant cytotoxic activity against KB (HeLa) or Jurkat T cells. The cytotoxicity of ajugasalicigenin against KB cells was remarkable (IC_{50} = 1.9 μM). Ajugasalioside C was the most active sterol glycoside against Jurkat T cells (IC_{50} = 3 μM) followed by the novel compound ajugasalicioside A (IC_{50} = 6 μM).

Ajugasalicioside A induced cell-cell contacts in Jurkat T cell populations similar to phorbol 12-myristate 13-acetate (PMA). To follow up this effect, the possible modulation of ajugasalicioside A on PMA-induced mRNA profiles in Jurkat T cells with reverse transcription real time PCR (RT-rt-PCR) was measured. The results suggested a NF-κB independent induction of cyclin D1 by ajugasaliciside A which indicates that this compound may stimulate differentiation processes.