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RESEARCH ON THE VALORISATION OF *CUSCUTA CAMPESTRIS* YUNCK SPECIES IN THE TREATMENT OF NEOPLASM

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Because classical medicine uses synthetic drugs to treat cancer, drugs which often have quite uncomfortable side effects, we set out to evaluate one of the remedies of traditional medicine – the species *Cuscuta campestris* Yunck (known in the popular language as "tortel", "torțel" or"borangic") – a parasitic plant harvested from *Medicago sativa* L. (lucerne).
The choice of subject was based on:
• information from some cancer patients beneficiaries of this treatment, regarding the traditional remedies;
• lack of relevant data from the specialized literature that justify their use;
• research of the chemical composition of the respective plant and the correlation between the chemical structure of the chemical constituents and the therapeutic action;
• verification of the therapeutic action on laboratory animals.
Objectives: therapeutic valorisation of *Cuscuta campestris* Yunck in the form of tincture and dry extract; identification and quantitative determination of the active principles for establishing the raw material quality; the influence of the host plant metabolism on the tortilla metabolism; verification of cytostatic properties; establishing the correlation between the chemical composition and the therapeutic action.
Material and methods: *Cuscuta sp.* ("tortel", "torch", "borangic") with flowers and fruits, harvested at full maturity from cultures of *Medicago sativa* L. – from Valea Lungă (Dâmbovița county), Alexandria and Rădoiești (Telorman county) and from spontaneous flora; seeds removed from tortelinfested grains.
Solvents used:water for infusion and 20% ethyl alcohol for maceration.
• *The verification of the identity* of the respective products was performed by: 1) *macro- and microscopic examination*; 2) extraction of active principles, identification and determination of their content – by classical methods, specific to each type of aglycone; 3) *the antimitotic action* – following the influence on the root elongation of the germinated seeds (*Triticum* test*, the* method prof. D. Constantinescu) and on the cytoskeletal actin F; on *rats with* Walker-Guerin *graft tumors*; *solutions to be analyzed*: 10% infusion and 10% extracts in diluted ethanol (20%), obtained from seeds of *Cuscuta campestris* (C), *Medicago sativa* (M) and their resulting mixture with trior (M + C), as well as polyholoside extract total obtained from the fruiting plant (P); 4) *acute toxicity* (LD 50) – performed on rats; 5) influence of host metabolism on *Cuscuta* metabolism – by determining the content in active principles of host and parasite; 6) deciphering the *mechanism of action* – correlating the chemical structure of the active principles identified with their therapeutic properties, quoted in the specialized literature.
Results and discussions. The aqueous and hydro-alcoholic solutions obtained from the seeds of *Cuscuta campestris* and *Medicago sativa* demonstrated: cytotoxic action on wheat roots (*Triticum vulgare* Mill.) – inhibits roots elongation, cell division (cytotoxic effect – stronger of aqueous solutions, probably by inhibition of cytoskeletal actin F) and retraction of protrusions. Both types of modifications confirm the antimitotic (cytostatic) action.
The identification of the same groups of active ingredients in the infusion and tincture of *Cuscuta campestris* Yunck, by chromatographic techniques (TLC, HPTLC, HPLC) confirms their effectiveness in the cancer treatment. We consider that an important role in the printing of the therapeutic action is played by: polyphenols (lignin derivatives by cytostatic and antioxidant action), carboxylic phenol acids and flavones (by hepatoprotective, anti-inflammatory, antioxidant, healing and diuretic action) and coumarin (by anticoagulant and antioxidant action).
Conclusions: The chemical composition of *Cuscuta* is different from that of its hosts. C*uscuta* is not toxic. This is why we believe that the *Cuscuta campestris* Yunck species can be successfully used in the treatment of cancer (if the host plant is not toxic).
Research will continue to determine the types of sensitive tumors. The influence of the host on *Cuscuta* 's metabolism is insignificant.

Keywords: neoplasm, cuscuta compestris, Yunck, tumors, phytotherapy.

INTRODUCTION

This work represents the valorisation of the researches carried out in the field of phytotherapy, at the Pharmacognosy – Phytochemistry – Phytotherapy Department of the Faculty of Pharmacy, University of Medicine and Pharmacy "Carol Davilla", Bucharest, for a period of more than 30 years, by several doctoral students, today professors (pH.d. Mihaela Dinu, pH.d. Robert Ancuceanu – Botany Department) or lecturers (dr. Ligia Duțu – Pharmacognosy – Phytochemistry – Phytotherapy Department) at the Faculty of Pharmacy, University of Medicine and Pharmacy "Carol Davilla", Bucharest.

The selection ofthis species for this research was based on its popular usageand aimed to determine the therapeutic quality by specifying the pharmacologically active constituents, the mechanism of action and the development of a pharmaceutical product, called ***Cusmed***.

Cancer is the general term that encompasses all the varieties of malignancies that are characterized by multistage genetic process, rapid and atypical growth, tendency to invade neighboring tissues and then to seed other regions of the body (metastases)[1].

The neoplastic cells are differentiated from the normal ones by: clonality, autonomy, anaplasia, metastasis. The transmission of malignant characters in the succession of generations of tumor cells, carrying multiple genetic lesions, accumulated successively and scattered throughout the genome, are strong arguments that support the genetic character of cancer[1, 2].

Mutational alterations of some of the genes that coordinate growth, proliferation, differentiation, apoptosis and maintaining genomic stability programs are lesions responsible for initiating, promoting and progressing the malignant process.

Genetic alterations that induce the malignant phenotype affect *genes* (allotypic, abnormal chromosome profiles are characteristic of most malignant cells) and the *genome* (as a whole). Polyploidy appears to be present in 99% of cancers, and genome instability is a defining feature of the cancer cell.

Because cancer has become a global health issue, it is difficult for people of all ages and chemotherapy to support it, due to multiple *adverse reactions*, so it was natural for various remedies to emerge. These determined the re-orientation of the specialists towards the evaluation of the traditional phytotherapeutic remedies[3-11, 20-25].

But without modern phytotherapy there could be no research. For example, before approaching the treatment of cancers with traditional remedies, a relevant choice must be made, based on the correlation between the chemical structure of the active principles present in the products to be used in the treatment of the patient and their therapeutic action, the previous verification of the action of the remedies selected for treatment (on experienced animals), the clarification of the content in active principles and monitoring of possible adverse reactions (RA).

I should mention that the research of products used in cancer phytotherapy was a major concern of the group of the Pharmacognosy, Phytochemistry and Phytotherapy Department, UMF "C. Davilla" Bucharest. The first researches in this field were initiated by prof. Eugeniu Constantinescu and collaborators, with the allantoin extracted from *Symphytum officinale* L. (popular named "tătăneasă"), on Walker-Guerin graft tumors in rats. The results were encouraging[17].

The diversity of cancer forms, triggers and determinants are essential elements in choosing the right remedies. The correlation of the chemical structure of the active principles in the selected remedies, with possible secondary reactions helps us to avoid them.

It is known that plants produce by biosynthesis a large variety of compounds (with the same basic chemical structure, or similar or different), which is why their action differs from that of pure chemicals, is more complex. That is why we should not trust all the recommendations of traditional medicine unless they are confirmed by chemical, pharmacological and toxicological research.

Usually, the research that is needed in such situations consists of: qualitative and quantitative determination of the chemical composition, the verification of the pharmacological properties, of the acute and chronic toxicity – through experiments on laboratory animals and possibly on volunteers, elaboration of a pharmaceutical product and its quality standard.

The selection of herbal products for cancer treatment should not be random. Each type or form of cancer has sensitivity to certain chemical constituents, classically called active principles. This is due to the different structure and optical isomerism of metabolites resulting from biosynthesis, which gives it a certain mechanism of action, such as:

- inhibition of the synthesis of nucleic acids (antimitotics) – colchicine, podophyllotoxin, vincristine, maitansins (binds to mitotic division spindle proteins, prevents polymerization of mitotic tube proteins and microtubule assembly, stabilizes them against depolymerization);

- other plant products inhibit DNA synthesis and replication by topoisomerases (I, II) – enzymes that intervene in DNA replication, through the ability to break and reconstitute them (camptothecin acts on topoisomerase I).

Many natural antitumor agents act as inhibitors of nucleic acid synthesis, but their mechanism is different:

- some are alkylating agents – by binding to electrophilic centers (electrophilic esters, α, β-unsaturated lactones, epoxides);
- others act selectively on thiols and enzymes that control cell division, probably by destroying electrophilic centers and microtubule assembly, resulting by-products having negligible carcinogenic activity; this is how cucurbitacins, bruceantine, tryptolide, elephantopine, baccharine act;
- there are active principles that inhibit DNA synthesis and replication by action on topoisomerase II (topoisomerases I and II intervene in DNA replication, act on the strands of the cell division spindle having the ability to break and reset them);
- other substances increase immunity – lectins from *Viscum album* L. (*Loranthaceae*) – mistletoe, semi-parasitic on various hosts and those of *Loranthus europaeus* Jacq. = oak mist (semi-parasitic on oak = *Quercus robur* and fir = *Abies alba* L.), and are effective in Hela, s arcom 180, bladder cancer;
- cis-stilbenic benzyl derivatives (raponticozide from *Rhaponticum carthamoides, Rheum raponticum, R. officinale* – resent) have chemical structures close to diethyl-stilbestrol; that is why they are estrogen, therefore effective in prostate cancer, but contraindicated in ovarian cancer; extracts of *Rhaponticum carthamoides* (*resent*), administered for 1 year in rats with brain tumors, induced with N-nitroethyl urea, had antitumor properties on tumors SNC-Bespalov *et al.*[8–9], as well as other active principles of various species[10];
- another mechanism by which some natural substances act as cytostatics, seems to be the balance between hydrophilicity and lipophilia of the respective substance.

From the reported examples, it appears that the mechanisms by which some plants act as cytostatic are diverse. Therefore the chemical composition, the properties of each active ingredient and any adverse reactions or other risks should be well known when using herbal remedies.

We draw attention to the tendency to generalize the treatment of cancer, regardless of its origin, with *products containing heteroids or other bis-benzyl-cis-stilbenic derivatives*, because the *efficacy of* these compounds is manifested only *in prostate cancer (through diethyl-stilbestrol released following hydrolysis). The generalization of this treatment to all forms of cancer is ineffective, sometimes even contraindicated, in ovarian cancer and hyperfoleculinaemia*[8, 12].

So, a good remedy for the treatment of a type of cancer cannot be considered as effective in any cancerous disease. Therefore, the information of traditional medicine must always be verified, more precisely the chemical composition of the respective product must be known, and then – based on the knowledge of the chemical structure of the active pharmacological constituents – we can predict the therapeutic action, adverse reactions or contraindications. Therefore, all the information must always be checked, before setting up a phyto-therapeutic treatment, I have been selected for neoplasm.

It is certain that the *generalization of the treatment of a certain type of cancer with a natural remedy, on all types of cancer, without chemical and pharmaco-toxicological researches, specifying the chemical composition, confirming the therapeutic action and possibly the mechanism of action, to draw attention to possible side effects and contraindications,* ***can fail*** *or* ***can put in danger*** *the life of the patient, thus aggravating the disease*. The information of *traditional* medicine *must be verified*.

An old Romanian proverb says: "who has no elders must buy them"! Because it is full of truth, we support it. Here is why. About 45 years ago I learned from a person (EC), operated on of breast cancer in Spitalul Județean Târgoviște (who had been informed by other patients with the same diagnosis), that an effective cure for cancer constitutes the "tortel" (*Cuscuta campestris*). She also followed that cure and survived the tumor extirpation for more than 25 years (she died of "old age" at the age of 85). Therefore, from the multitude of species of this genus, we have selected for research *Cuscuta campestris* Yunck (tortel) harvested at full maturity, a species that parasitizes numerous crop plants or sponges, to verify the *dose* of *truth* or of *legend*.

Because in the specialized literature consulted I did not encounter any profile research at that time, to confirm these actions, as a pharmacognost I considered that the topic would be of national interest for research, especially for doctoral students, but also for dedicated oncologists. In

doing so, I had the great pleasure of collaborating with a few former students, now teachers (Mihaela Dinu, Ligia Duțu, Robert Viorel Ancuceanu) at the University of Medicine and Pharmacy "Carol Davila", Bucharest, Faculty of Pharmacy, from the time of their studies and after the preparation of their doctorate, as well as with some dedicated specialists in the field of oncology (Professor I. Bălănescu and his collaborators from the Oncological Institute of Bucharest)[31-35, 37].

For the seriousness and depth with which they have approached the research (chemical, pharmacological and toxicological), for their contribution to the knowledge of the active principles of *Cuscuta* traditionally used in cancer therapy and to decipher the mechanism of action, or to check their action on animals with Walker graft tumors. Gueren, I thank to all of the contributors and I am deeply grateful to them.

The objectives of our research consisted of:

- determining the quality of the extracts obtained from *Cuscuta campestris* Yunck. and from *Medicago sativa* L. by identifying and dosing the active ingredients;
- verification of the therapeutic action *in vitro* and *in vivo*, on cultures of Hep G2 malignant hepatocytes, murine ascitogen EL 4 (malignant thymocytes), B16 melanoma in mouse and rat, Walker-Guerin graft tumor collected from mouse ascites inoculated with the respective tumor;
- specifying the mechanism of action and any possible adverse reactions;
- *obtaining a tincture or dry extract* of the whole plant (*grass*) and of the seeds of the species *Cuscuta campestris* Yunck separated by trior (s *emen*) (Cusmed – provisional name), with cytostatic action;
- checking their quality by identifying the active principles, starting from the correlation metabolism – phylogeny and chemical structure – therapeutic action; the experiments were performed on: plant cells, animal cells, animals with graft tumors and on 1 female volunteer with bladder cancer, after surgical removal.

The research consisted of:

1. *verification of the identity* and *quality of* the *Cuscutae campestris herba* product by macro- and microscopic examination, in order to establish the specific morpho-anatomical characters;

2. *extraction of the active principles is* by infusion of water and soaking with 20% ethyl alcohol, the choice of solvent and extraction method;

3. *determining the quality of the extracts* obtained – by qualitative and quantitative chemical analysis of the active principles (flavones, phenol-carboxylic acids = AFC, coumarins, carotenoids), according to the pharmacognostic analysis elaborated by Prof. dr. Doc. Eugeniu Constantinescu *et al.*[16];

4. *evaluation of acute toxicity* – LD 50 in rats (9);

5. *evaluation of cytostatic action* on: *vegetable cells* – germinal wheat root (*Triticum vulgare* Mill.) – phytobiological method D. Constantinescu (16); normal animal cells (*true*); thymoma cells EL 4; Walker ascites cells 256; *in vivo assays in* rats with Walker-Guerin graft tumors 256, B 16, EL 4; *the influence of the* extracts on certain *blood biochemical constants*[8-10, 19-20];

6. evaluation of cytostatic action on a volunteer (female) with bladder tumor, surgically removed on December 2018, in order to specify: type of cancer, mechanism of action and adverse reactions; setting the pharmaceutical form and dosage; obtaining a pharmaceutical product provisionally called *Cusmed* (20% tincture).

MATERIAL AND METHODS

From the multitude of traditional remedies used in the treatment of cancer, we selected a species commonly found in our country – *Cuscuta campestris* Yunck, especially researched for its removal from various cultures of food, medicinal, decorative, fodder plants, known for the damages they produce.

The genus *Cuscuta* presents numerous parasitic species and varieties, popularly called "**tortel**", "torțel" or "borangic" (due to the bushy appearance of the thin, yellow stems, which are interwoven into buds). They are spread on numerous crop species (lucerne, clover, potato, vegetable, vegetable, ornamental) or spontaneous, from all over the world. Of these, we list only a few:

• *C. campestris* Yunck the varieties: *typica* form *orşoviana* Bui of; *breviloba* Buia; *minor* Dark; *moldavica* Buia with ssp. *pentagona* (Engelm), var. *pentagon* and lime. *transilvanica* Buia;

• *C. trifolii* Babington var. *angustifolia* (Engelheim) Buia – the forms *breviscoama* and *longiscuama*;

• *C. europaea* L., *C. planiflora* Tenore, *C. aproximata* Babington, *C. epithymum* (L.) Murray, *C. monogyna* Vahl., *C. pentagona* Engelm var., *C. lupuliformis* Krock, *C. prodani* Buia, *C alba* Presl, *C. epilinum* Weihe)[39].

They parasitize multiple crop plants, belonging to several families: *Apia tea, Asteraceae, Chenopodiaceae, Cruciferae, Fabaceae, Poaceae, Rosaceae, Solanaceae*.

The species of the genus *Cuscuta grow* on numerous plants, ruderal or of culture, frequently being found on:

- fodder (*Medicago sativa* L. = alfalfa and *Trifolium sp.* = Trif sheep);
- vegetables and vegetables (*Allium cepa* L. = onion, *Allium ursinum* L. = garlic, *Capsicum annum* L. = pepper, *Cucumis sativus* L. = cucumber, *Cucurbita pepo* L. = melon, *Petroselinum crispum* (Mill.) Nyman ex AW Hill = *Petroselimum sativum* Hoff. = *Petr oselinum hortense* auct. = Parsley, *Daucus carota* . L. = carrot, *Solanum tuberosum* L. = potato, *Solanum melongena* L. = eggplant);
- Ornamental plants (*Cosmos bipinatus* = butterfly, meadow; *Dahlia variabilis* = dahlia, *Petunia sp.* = *Petunia*, *Zinna sp.* = Hornbill);
- medicinal plants (*Arctium lappa* = brusture, *Plantago sp.* = Patlagina, *Matricaria chamomilla* = *chamomile*) and ruderal (*Atriplex oblongifolium* = lobode, *Amaranthus sp.* = News, *Sonchus sp.* = Susai)[11].

The variety of the species of the genus *Cuscuta* and its hosts[26-35] determined us to limit our research only to the species *Cuscuta campestris* Yunck, harvested at full maturity, from crops of *Medicago sativa* L. (lucerne), separated or not from the host plant, coming from Alexandria (Teleorman county) and Valea Lungă (Dâmbovița county).

Although the "torțel" is considered a parasitic plant, I tend to believe that the species is semi-parasitic, at least in the first stage of life, because it fixes its haustors in the woody vessels of the host through which it has access to raw sap (not in the Liberian ones, with elaborate sap – as parasitic plants), and the chlorophyll of the leaves from the first stage of development certainly intervenes in the biosynthesis of some nutrients necessary for its survival, even different from those of its hosts. Therefore, we assume that the way of life of the tortel is autotroph first, then mixed, because it produces by foreign biosynthesis compounds of the host plant.

For ***Cuscuta campestris*** Yunck literature mentions two *subspecies*:

• **ssp. *Campestris*** – with the *typical* Buia varieties (stamens shorter than the lobes of the corolla); *typica f. orşoviana* Buia (stamens of the same length with corolla lobes and subulate filaments); *brevifolia* Buia (flowers arranged in lax glomeruli and corolla lobes longer or equal to the width of the base); *minor* Buia (flowers 2–2.5 mm long, arranged in compact glomeruli); *moldavica* Buia (flowers 3–3.5 mm long, arranged in compact glomeruli, larger than 10 mm);

• **ssp. *Pentagon a*** (Engelm) with the variety *pentagon* a[27].

The successful use of this species for therapeutic purposes, the few profile researches and the prolongation of the life of many of the cancer patients, who have used this remedy, have determined us to approach the search for cuscus.

As the therapeutic action is printed by the active principles (constituents with certain chemical structures, which correspond to actions common to the group, or specific to a species), we set out to evaluate the therapeutic potential of the above mentioned species.

In order to obtain an active pharmacological product (the main objective of this paper), the research consisted of:

1. *Establishing the identity of the* raw material (using the macro- and microscopic characters) – according to the pharmacognostic analysis, that the products are easily identifiable[13-16];

2. *The quality control of the raw material* (*grass* and *semen*) was carried out on the extractive solutions obtained from the whole plant or from the seeds resulting in triorum, by successive extraction with solvents of different polarities (petroleum ether, ethyl ether, ethyl acetate, ethanol 10 and 20%) or by maceration in water of the seeds previously extracted in ethyl acetate, and consisted in the identification of the active principles (flavones, phenol-carboxylic acids, coumarins, polyholosides), by specific chemical reactions, chromatographic investigations (thin layer chromatography = CSS, high performance thin layer chromatography = HPTLC; mass spectrum gas chromatography = GC/MS) and by quantitative determination. For this purpose, methods cited in the specialized literature were used[12, 14];

• The separation of active principles was performed by thin layer chromatography (CSS) and high performance liquid chromatography (HPLC), in the Camang horizontal development chamber (HPTLC) using reference substances (quercetol, kaempferol, rutoside);

• the solutions to be analyzed were obtained by refluxing in the Soxhlet apparatus, 5 g of plant product (grass with seeds = h, or seeds separated at thi = s, from *Cuscuta* and from the host plant - *Medicago sativa*), with 100 mL solvent (20% alcohol or water); the solutions obtained in water from grass with seeds were coded Sah and those from seeds Sas; those obtained in 20% ethanol (tincture) from grass with seeds = Seh and those from seeds = Ses;

• analysis method – *thin layer chromatography* (TLC);

• mobile phase (v / vv / v): ethyl acetate - formic acid - water, 80: 8: 12; propanol - ethyl acetate - water, 40:40:30; ethyl acetate - acetone - water, 80:80:20 (top layer);

• reference substances: 0.01% solutions of quercetol, kaempferol, hesperitol, diosmin, rutoside and naringenol in ethyl acetate;

• reagents: UV (ultra violet), KOH – 10% aqueous solution, *p*-nitroaniline diazoted (Pauly reagent);

• solvents of different polarities (ether, ethyl acetate, 20% ethanol, water);

3. The *choice of the solvent suitable for the extraction of the active principles* was possible due to the testing of solvents with different polarities (ethyl acetate, water, 10 and 20% alcohol), followed by the identification and quantitative determination of the active principles (flavones, phenol-carboxylic acids, coumarin), by specific methods, cited in the specialized literature[13, 17-18];

4. *Obtaining extracts* (infusion, tincture, dry extract) and qualitative and quantitative determination of the content in active principles, in order to choose the extraction method;

5. Toxicity determination – by determining LD 50 in rats.

6. The *cytostatic action* was *tested* on the plant cell, respectively on the germinated wheat roots (D. Constantinescu method), on rats with Walker-Gueren graft tumors[17, 41];

7. The *testing of the pharmacological properties* was performed on laboratory animals and on a volunteer (patient with surgically removed bladder tumor), based on the relationship between the chemical structure of the active principles and their pharmacological action.

All of these researches looked at the therapeutic efficacy and the possible adverse reactions.

In order to reach these objectives we used methods cited in the literature.

On this occasion we thank Prof. Dr. I. Bălănescu and his colleagues from the Oncological Institute of Bucharest, for supporting our research, through experiments on rats.

RESULTS AND DISCUSSIONS

I. The identity of the investigated **species** was confirmed by *the macro- and microscopic characters* observed on *the vegetal material* we searched, which correspond to those mentioned in the specialized literature for *Cuscuta campestris* Yunck and respectively for *Medicago sativa* L.

1. *CUSCUTA CAMPESTRIS* YUNCK

• Macroscopic characters: filiform stalk with a diameter of 0.7–0.8 mm, yellow-orange, rarely greenish, branched forming nets around the host plant, often including the neighboring specimens, with cymorose or umbeliform inflorescences, made up of 10–30 pentamere flowers. The calyx is cupuliform, with straight, lobed lobes. The campanulate corolla has triangular lobes or ovates, reflexes. The stamens attached between the lobes of the corolla are erect, with scales at the lower part forming a tube with ovate lobes and furred edges. The gin is made up of a globular ovary, with two styles equal or shorter than the globular ovary. The stigmas are mottled. The fruit is a flattened capsule, with inter-stellar dehiscence and contains 2-4 ovoid seeds, brown or brown (Fig. 1). These coincide with the characters mentioned in the specialized literature for *Cuscuta campestris* Yunck, Which has the *subspecies campestris* (Buia) and *pentagon* (Engelm) and several varieties:

• *Cuscuta campestris* Yunck subspecia *campestris* Buia comprises the varieties *typical of* Buia (with stamens shorter than the lobes of the corolla) and *typical of the Orsoviana* Buia (with stamens of the same length as the lobes of the corolla, subulate filaments);

• Cuncuta *campestris* Yunck subspecies *brevifolia* (Buia) has flowers arranged in lax glomeruli, lobes of corolla longer or equal to the width of their base, shows *minor* varieties Buia (with flowers of 2–2.5 mm long arranged in small compact glomeruli) and subspecies *moldavica* Buia (with 3–3,5 mm long flowers arranged in compact glomeruli, larger than 10 mm)[2, 39].

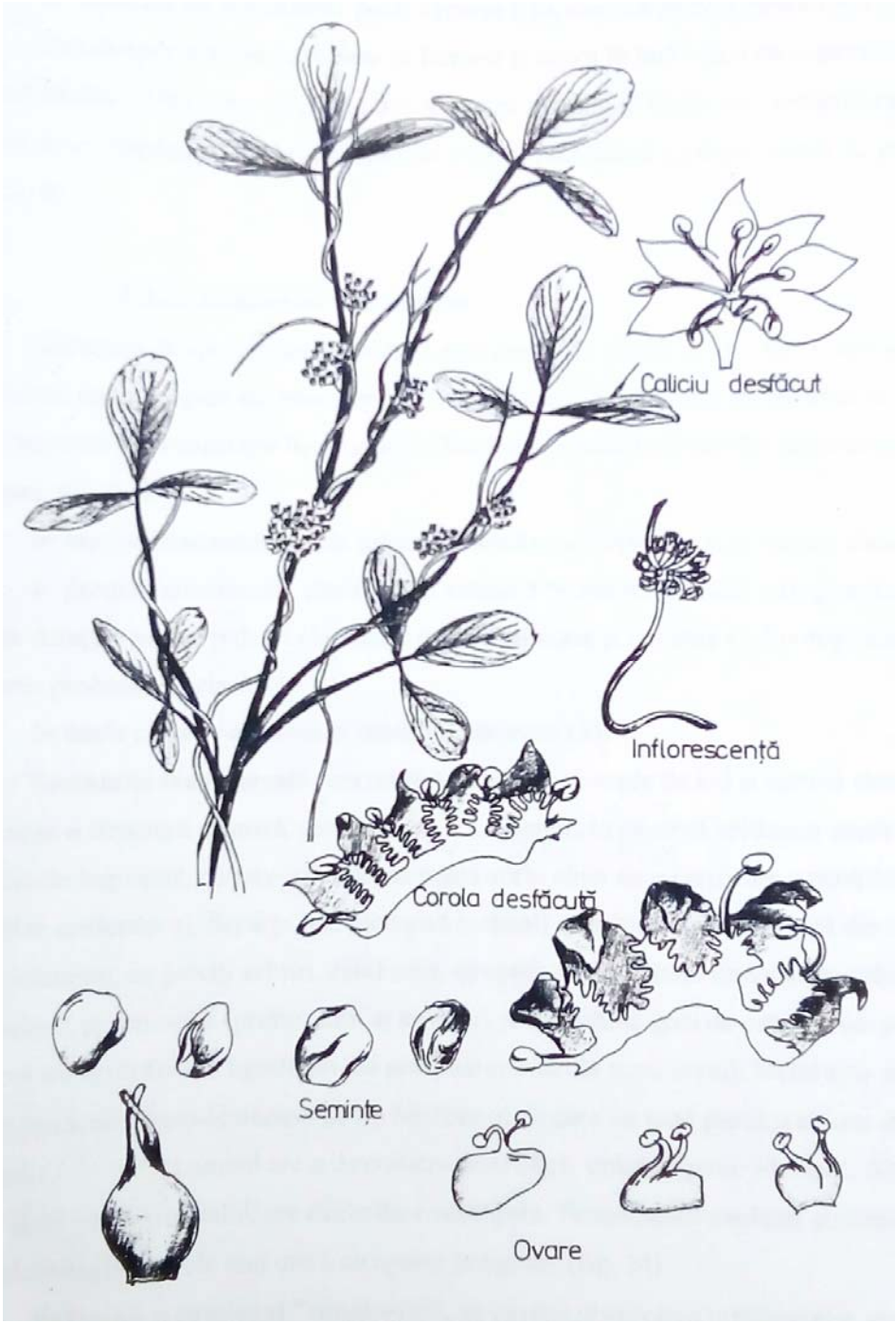

Figure 1. *Cuscuta campestris* Yunk – macroscopic character.
(Ph. D. thesis, pharmacist Mihaela Dinu, p. 63, fig. 23).

• *Microscopic characters*

• The cross section (double staining with carmine and iodine green) through the strain of the species *Cuscuta campestris* Yunck presents the primary monostelical structure characterized by: tetrahedral contour, single-layered epidermis, protected by a thickened cuticle, slightly cerified and rarely stomata; developed cortical parenchyma (composed of large cells, rounded, with thin walls, cellulose and intercellular spaces), in which 6 bundles of wood are distributed, protected by a colenchymal danger. The endoderma and the pericycle are difficult to distinguish from the surrounding cells. The star is made up of 6 bicolateral bundles, in which paradoxically the xylem (5–6 wood vessels) is developed, with centrifugal development, typical of the stem. The free (phloem), disposed on one side and the other of the xylem (wood), has

centripetal development. The medullary parenchyma and the medullary rays are made up of smaller cells with a polygonal outline. The developed wood supports the hypothesis of *Cuscut*'s mixed lifestyle, at least in the first stage of development, an observation not yet reported in the specialized literature.

• The powder is characterized by: elongated epidermal cells, with moniliform thickening – from the sepals; stomata with 3–4 attached cells and cuticular strips – from the corolla; fragments of small-scale wood vessels, fibers and parenchyma with starch – from the stem; epidermis with elongated cells and thin walls – from the stem filament; endothelial fragments with lignin bands, papillae on the corolla and stigma.

Although the literature mentions *Cushions* as having a parasitic life, the *presence of wood vessels and gutters implanted in the host's woody vessels* (not free – as in parasitic plants), causes me to state that *the lifestyle of the cushions is mixed* (autotrophic – after germination), at the beginning of the development and parasitic period – after the meeting with autotrophic plants).

The microscopic characters (the elongated epidermal cells on the stem filament, or the polyhedral ones with cuticular strips on the carpal and endothelium, the papillae on the corolla, the endosperm with epidermal cells whose walls are thickened and contain reserve substances) *confirm the identity of the* species *Cuscuta campestris* Yunck.

2. *MEDICAGO SATIVA* L.

• *Macroscopic characters*: erect, ascending, 4-edged, slightly pubescent stalk; leaves composed of 5 ovate-cone leaflets, gamosepal calyx, blue-violet fennel-type corolla, bent outwards, longer than the wing and keel, androceu diadelf (nine stamens united and one free); monocarpelary gynoecium, with short pedicellate ovary, shorter in style than the ovary, in the form of a sword, bent outwards and bilobed stigma. The stem and leaves are green on both sides, the flowers are blue-purple. The taste is bitter-astringent, slightly fragrant, characteristic odor. The powder is irritating to the ocular and nasal mucosa.

• *Microscopic characters* – cross-section through the stalk (double staining – iodine green and carmine tarnished): primary structure, monostellar, with tetrahedral contour or beginning of secondary structure; uni-stratified epidermis made up of cells whose cuticle is thickened, slightly cerified, with rare stomata; weakly developed cortical parenchyma, composed of large cells, rounded, with thin walls, cellulose and intercellular spaces; star made up of 6 light, two-sided open beams, bordered by sclerified pericyclic fiber bundles; wide medullary rays and highly developed medullary parenchyma; the endoderma and the pericycle are difficult to distinguish from the surrounding cells; the medullary parenchyma and the medullary rays are composed of smaller cells with a polygonal outline.

• In powder it is observed: epidermis with moniliform thickening – from the sepals; epidermal cells with 3–4 attached cells – from the corolla; parenchyma with starch, wood vessels of small caliber – from the stem and fibers – from the calyx (Fig. 3).

The elongated epidermal cells (from the stem filament), or the polyhedral ones with cuticular striations (from the carpal), the papillae on the corolla, the endothelium, the endosperm with epidermal cells whose walls are thickened and contain reserve substances, confirmed the identity of the species (*Cuscuta campestris* Yunck).

II. The quality of the plant products was verified by: *chemical reactions specific to* the active principles, chromatographic research and *quantitative determinations of active principles*.

The solutions to be analyzed were obtained by successive extractions with *solvents of different polarities* (petroleum ether, ethyl ether, ethyl acetate, ethanol) – in the Soxhlet apparatus; by soaking in 20% alcohol, or by infusion with water.

1. IN *CUSCUTA CAMPESTRIS* YUNCK

Through specific chemical reactions, the following were identified:

• sterol and triterpenic compounds – free (phytosterols, phytosterols) and glycosides; saponozides, carotenoids, hydrocarbons, lipids, flavonic and flavonozide aglycones, phenol-carboxylic acids (AFC) = *o* -dihydroxy-phenols (ODP), lignans, coumarins, glycorezines[3, 4];

• aromatic compounds (probably coumarin) and flavones (of which quercetol and kaempferol were identified) – from the polyphenolic fraction, by HPTLC (high performance thin layer chromatography), in the CAMAG development room;

• numerous alkanes, lower fatty acids (without pharmacological importance), higher fatty acids (palmitic, stearic) – from the lipophilic fraction, obtained by extraction with ethyl acetate;

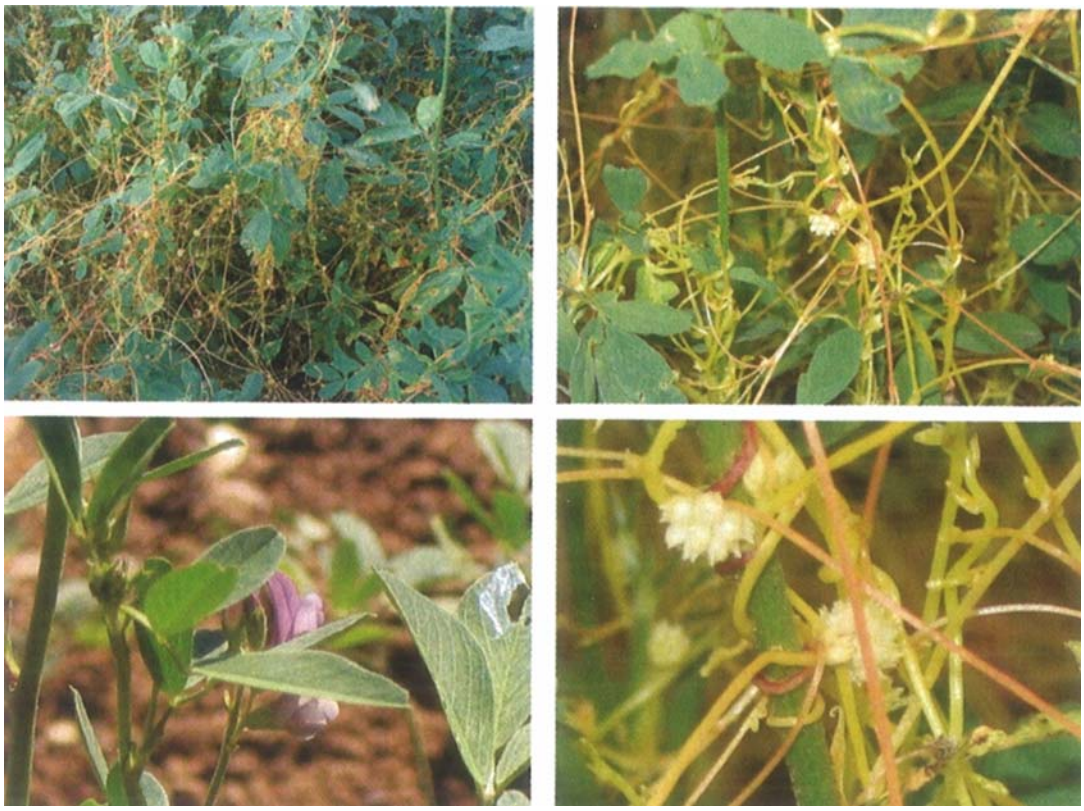

Figure 2. *Medicagos sativa* parasitic by *Cuscuta campestris* Yunk
(Ph. D. thesis, pharmacist Robert Ancuceanu, pag. 48, fig. 38).

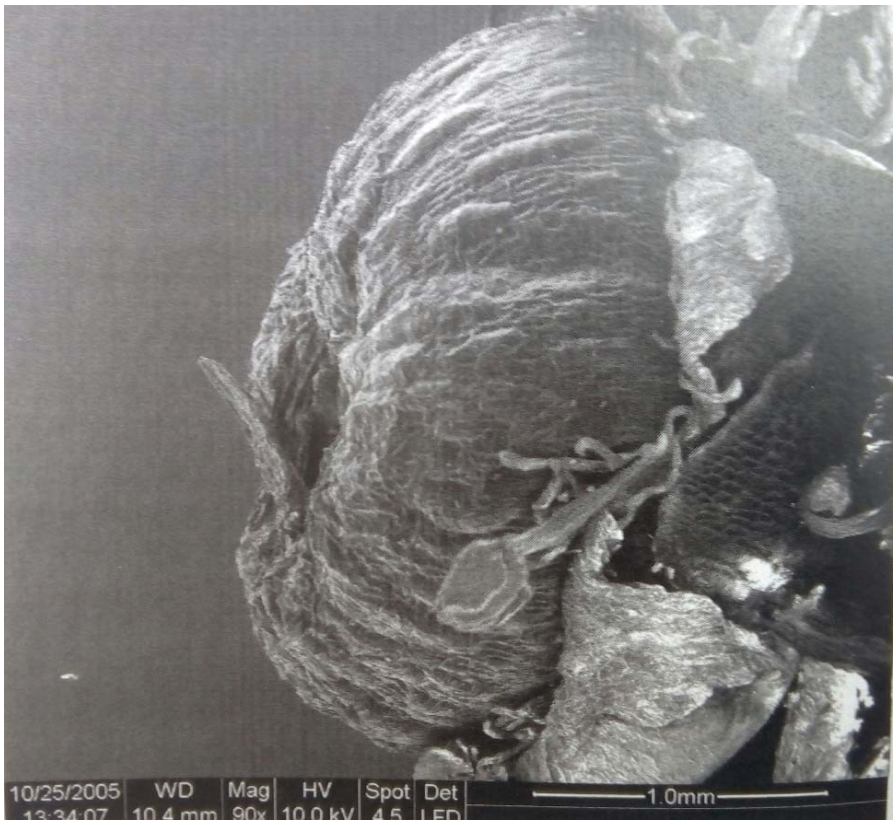

Figure 3. *Cuscuta campestric* Yunk – microscopic image.
(Ph. D. thesis, pharmacist Robert Ancuceanu, p. 40, fig. 13).

• lignans - obtained by extraction with acidified dioxane with HCl (J. Grus – 1921, B. Holmberg – 1934, FE Brauns and H. Hibbert – 1935, Freudenberg, 1954) and fractional precipitation with benzene (J. Desmet, 1960), according to the procedure described by C. Bodea et al. (10); of the spots that were colored red with hydrochloric fluoroglucin, two (Rf = 0.76, 0, 82) were considered as possible lignans;

The quantitative determination of the active principles has led to the following results:

• 0.63 g% water vapor entraining compounds (of which hydrocarbons consisting of 13–16 carbon atoms with branched chain have been identified), oxygenated compounds of some hydrocarbons (probably degradation products) and esters;

• flavones (complexed with AlCl 3), expressed in g quercetol = 0.12–0.15% in *Cuscutae herb*: 0.08–0.10 g / 100 mL 10% aqueous extraction solution and 0.082–0.086 in tincture 10%;

• phenol-carboxylic acids (Arnow photo-colorimetric method), expressed in g% caffeic acid: 0.04838–0.09511 in *Cuscutae herba* and 0.00454–0.00605 in 10% tincture;

• coumarins and derivatives (Roberts and Links photo-colorimetric method) – expressed in g% coumarin: 0.60–0.65 in the vegetable product; 0.0993–0.158 in 10% aqueous extract and 0.426–0.431 in dye;

• the saponozide content (0.95–1.05 g%) was also evaluated by the foaming index (I = 133) and the hemolytic index (IH = 416–500), variable depending on the origin of the plant product;

• polyholosides were extracted with different solvents (cold and hot water, potassium oxalate, hydrochloric acid); the resulting solutions were purified by precipitation in alcohol or acetone; the resulting precipitate was washed with alcohol, dissolved in water and re-precipitated in alcohol.

GC/SM analysis (gas chromatography coupled with mass spectrometry) **of the extract** obtained in **ethyl** acetate from *Cuscuta* led to the identification of the following constituents: fatty acids (butyric, palmitic and stearic), aromatic acids (*p*-hydroxy-benzoic, vanilic); esters (1-glyceryl acetate, glyceryl-triacetate, bis-ethyl-hexyl phthalate, acetyl-sterol), hydrocarbons (tricosan, pentacosan, nonacosan, decane, pentatriacontane) and higher aldehydes, along with numerous higher alkanes without pharmacological importance and bis-ethyl-hexyl phthalate; To a lesser extent, esters (1-glyceryl acetate, glyceryl-triacetate, bis-ethyl-hexyl phthalate, acetyl-sterol) and higher aldehydes were identified.

Of these, the *types of higher alkanes and carboxylic acids have not been reported in the literature as being present in dry C species.*

GC/MS of **fraction entrained with the steam** of *Cuscuta* showed the presence of 0.63% trainable compounds consisting of hydrocarbons (2-methyl-6-ethyl-decane); alcohols (1,3-buten-diol, 2,6-diterbutyl-1-hydroxy-toluene); ketone (hexa-hydroxy-farnesyl-acetone); ethers (di-iso-propoxy-methane, 4-isopropoxy-butanol); esters (bis-2-methoxy-ethyl-phthalate, 3,7-dimethyl-6-octen-butyrate) – not of pharmacological importance.

Because the structures of the identified volatile compounds do not correspond to the structure of the constituents of the volatile oils (mono- or sesquiterpenic, alveolar or cyclic), we deduce that *the respective fraction is not composed of volatile oil*, a fact confirmed by: unpleasant odor, lack of specialized histological formations (peri glans, secretory bags or channels – characteristic of plant products containing volatile oil) and of small quantities of oxygenated compounds.

Comparing the results obtained in the analysis of the two fractions, we find great differences regarding the chemical structure of the identified compounds; *hydrocarbons from the fraction obtained by water vapor entrainment are not specific to volatile oils, from which we deduce that they could be constituents of the heavens.*

From **glicorezina** isolated from *Cuscutae herba,* dissolved in the alcohol by **gas chromatography coupled with mass spectrometry** (GC / MS) were separated and identified fatty acids (stearic, linoleic acid, arachic acid, behenic acid) ester 7-methyl-nonanoic, alcohols (n-octadecanol, n-nonadecanol), sterols (campesterol, stigmasterol, γ-sitosterol), oases (rhamnose, glucose, mannose, D-glucosamine) and so on. a.

Following the examinations performed, it was concluded that the *glycerine is composed* of esters of 11-hydroxy-palmitic acid (as aglycone); rhamnose, mannose, glucose and glucosamine (as a carbohydrate); the hydroxyl groups of the oases are partially esterified with acetic and butyric acids.

In the solution obtained from the **seeds,** by refluxing with ethyl acetate, using chromatography on silica gel plates 60 F 254, coumarin derivatives and flavones were detected.

Research should be continued to: determine the pharmacologically-active dose; detection of any adverse reactions; setting of the pharmaceutical form and dosage of administration.

2. *Medicago sativa* contains:

• alcohols – trifoliol, lucernol, medicagol, sativol;

• simple oases (glucose, fructose);

• diglycosides – 4-β-[D-manopyranosyl]-D-manopyranose; 4-β-[D-galacto-pyranosyl]-D-manopyranose, 6 α-[D-galactopyranosyl]-D-manopyranose;

• triglucoside – 4 [βD- manano-pyranosyl -4-(βD-manopyranisyl] -D-manopyranose];

• 10–12% pectic acid polyholosides in leaves and 7–9% in seeds;

• coumarins - esculetol, scopoletol, dafnoretin;

• cumestanes-cumestrol, 3-methoxy-cumestrol, 4-O-methyl-cumestrol, 11,12-dimethoxy-7-hydroxy-cumestrol;

• nitrogen compounds – alkaloids and non-alkaloids

• alcohols – trifoliol, lucernol, medicagol, sativol[26].

The quality of the ***Medicago sativa*** product is characterized by:

• 5.53–8.05 g% ash total and 1.48–1.82 g% ash insoluble in hydrochloric acid;

• 27.25–32.67 g% water soluble substances;

• 2.98–5.38 g% pectic acid polyholosides – in leaves and 7–9% – in seeds;

• 195–253.8 mg% flavones expressed in rutoside;

• 180.4–256.6 mg% tannin expressed in tannic acid;

• 245.8–375.5 mg% total caffeic acid polyphenols[23, 26];

• 524–1088 mg% saponozide;

• 16.28–20.39 mg% carotenoids expressed in β-carotene;

• 4206–5,579 haemolytic compounds; hemolytic index = 463.82–557.10.

III. Acute toxicity (LD 50) – determined in rats

• In *Cuscuta campestris it* is 4500 mg / kg body weight, equivalent to 420 g of substance for an adult of 70 kg[5]. The body weight of the rats did not undergo statistically significant changes. So 10% tincture and dry extract are well tolerated by rats and people, do not put the patient in overdose (they are not toxic).

• *Medicago sativa* is 6000 mg / kg body weight, equivalent to 420 g of substance administered to an adult weighing 70 kg.

IV. The evaluation of the pharmacological properties was performed on: the plant cell, rats and voluntary patients with breast cancer and haemorrhagic bladder tumor (after surgical removal) – by administration of **Cusmed** po (10% tincture) and infusion.

V. The antiproliferative effect of the 20% alcohol extracts (v / v) obtained from the mixture of the seeds of *Cuscuta campestris* Yunck and *Medicago sativa* L. (from the Slobozia decuscitation station), was tested in 8 increasing dilutions – in RPMI medium (1 / 1, 1/10, 1/50, 1/100, 1/500, 1 / 5,000, 1 / 10,000, 1 / 100,000), on *thymoma cells dead in ascitogen, IL-2 secretory* (EL-4), obtained from mouse ascites inoculated with the respective tumor.

It was found that *the cytotoxic effect* appears only in the 1/1 solution (not diluted).

• **Cyto-toxicity** of the strain of *Cuscuta campestris* Yunck. collected from a mixture of *species from the spontaneous flora revealed an inhibitory IC 50 concentration of 35μg / mL on Hep G2 cells.*

• In animals inoculated with *Walker carcinoma 256* and treated with *Cuscuta* extract, a slower evolution of tumor development was observed. Over tumors of different sizes, early tumor stagnation or very slow evolution and faster growth of large tumors were noted. The values of tumor volume were not normally distributed for batches *Cuscuta* S and *Medicago.* The change in body mass was not significant. Survival at 120 days was 23.07%, compared to the control group where all animals died.

The results of the extracts obtained from Cuscuta were inferior to the extracts obtained from Medicago, where the survival was about 43%.

• Research on *melanoma B 16* was performed on mice belonging to line C-57 B1 / 6, females aged 10–12 weeks, with body mass of 18–22 g, inoculated by daily intra-tumoral injection with 8 × 10 5 g and determination of the tumor volume on days 10th and 15th of the inoculation. There were 5 groups of animals (4 sample groups of 5 animals and 1 control group of 10 animals), which were given daily *hydro*-alcoholic extract of *Cuscutae* intra-tumoral *herb*, starting from the tenth (group C 10) and on the fifteenth day after inoculation and (group C 15), or *Cusmed* 1: 1, intra- *tumorally*, starting on the tenth and 15th day after inoculation (groups CM 10, CM 15). The assessment was reported to the untreated witness.

The tumor is palpable in all animals from day 7.

Based on the observations made over a period of 32 days, the following conclusions were drawn:

• on the *murine* tumor tumor line EL-4, under *in vitro* culture conditions, the results indicate the *presence of an anti-proliferative and cytotoxic effect of the extract, but the results do not offer a dose-response curve, but an all-or-nothing action, the effects being recorded only at 1/1 dilution (whole extract);*

• *The effect of extracts obtained from Cuscutae campestris grass is slightly different on Walker 256 ascites cells, depending on the solvent used for the extraction of the active principles and the contact time.* From this we deduced that the most active constituents are the hydrophilic ones (phenolic*oxyhydroxy* acids = ODP, also called phenol-carboxylic acids = AFC; flavonozides and saponozides).

• On *melanoma B16* (solid tumor in the mouse) *it has no significant effects*, it does not produce different survival time compared to the control group, although there is a slight effect of reducing the volume of tumors, thus a *slight influence of Cuscuta campestris on melanoma B16*.

Cuscutae herba fluid extract and dry extract (Cusmed) showed no antitumor action on the human malignant ascitogen EL 4 and melanoma B16.

VI. The determination of the extraction process of the active principles consisted in choosing the appropriate solvent for the extraction of the active principles. For this purpose extractions were performed with water (by infusion) and with ethanol (by maceration), in a concentration of 10 and 20%. In the extractive solutions, the active principles (flavones, tannins, phenol-carboxylic acids, coumarin) were identified and dosed. The solvent that extracted the greatest amount of active ingredients was used to obtain the extract.

The identification of the chemical composition was carried out by the method elaborated by E. Constantinescu *et al.*[16], the focus being on the main groups of active substances soluble in dilute alcohol (10 and 20%) and in water, with possible therapeutic potential in different forms of cancer (mentioned in the literature).

VII. The quality of the raw material was evaluated by determining the content of pharmacologically active substances, in this case – phenol-carboxylic acids (AFC), flavones, coumarin –, by colorimetric methods[16] and gas chromatography coupled with mass spectrometry[13], comparatively with said reference substances.

The evaluation was based on the previously constructed *standard curve*, with reference substances in different quantities, because the color intensity is directly proportional to the concentration. The reagents used to measure the active ingredients were:

• *aluminum chloride* – for *flavones* (rutoside, quercetol), with which they form complexes colored in yellow, soluble in water;

• *Nitric acid* – for phenolic – *carboxylic acids* (caffeine or chlorogenic) with which it forms unstable iso-nitrosoderivatives; they spontaneously isomerize to oxime; oximes, due to their weak acid character, dissolve in alkaline solutions, giving red derivatives.

Because the intensity of the coloration is directly proportional to the concentration in active principles, their quantity can be calculated by interpolating the extinction in the *standard curves*, previously constructed with known quantities of rutoside or quercetol – for flavones; caffeic or chlorogenic acid – for phenol-carboxylic acids, coumarin - for coumarin derivatives.

The verification of the chemical structure was performed using mass spectrometry (MS) and nuclear magnetic resonance (NMR), compared to reference substances.

VIII. Choice of solvent and extraction method

The extraction was performed by: water infusion for 30 minutes (solution A); maceration for 8 days with 10% ethanol – (solution B). The respective solutions were separated from the plant product by filtration (filter paper). Their quality was appreciated by the content in active principles.

IX. The quality of the extracts obtained consisted in the determination of the content in pharmacologically active substances (flavones, phenol-carboxylic acids), as well as in pharmaco-toxicological determinations (LD 50 and antimitotic activity)[36].

Determination of water content by drying in the oven – according to FR X.

The quantitative determination of the *flavones* was performed colorimetrically, by chelating with aluminum chloride; the color intensity of the formed chelate was evaluated against the standard curve previously constructed with a reference substance (usually rutoside or quercetol).

Quantitative determination of their carboxylic phenol acids was performed by diazotization (with NaNO 2 in HCl medium) and measuring the absorbance of the resulting compound at $\lambda = 510$ nm.

X. The objectives of the **drug-toxicological research** were several determinations:

• *Acute toxicity* DL 50 (dose to be administered in pharmacodynamic studies), *chronic toxicity* and *subacute toxicity* were determined in the mouse by administration of the extracts at different concentrations (to detect the tolerability of the respective substance in several administrations)[30, 35]

• testing of pharmacological actions was performed on the normal plant and animal cell[20-22];

• testing the *anti-proliferative effect* – on *thymoma* cells *EL 4, melanoma B16 and ascites cells Walker-Guerin 256, breast cancer* by methods cited in the literature[8, 9].

RESULTS

1. *Determination of acute toxicity DL 50* – in Wistar white male mice, NMRI strain (FR X) showed: lack of toxicity of aqueous and hydroalcoholic extracts; the body weight of the animals did not change statistically significantly at higher doses (1500–4500 mg / kg body), which allows their administration without precautions; lethality appeared upon administration of 4510 mg / kg body weight[7].

2. *Antimitotic action* was *determined* first on plant cells (D. Gr. Constantinescu method) and animal cells HepG2 (hepato-cellular carcinoma), then on rats – by inoculation with solid tumors (Walker-Guerin 256).

The evaluation was performed on tumor cells of rhabdo-myosarcoma (RD) and melanoma B 12, compared with normal cell cultures (vera).

Based on the results obtained previously, a volunteer with surgically excised bladder tumor was refused, who refused the chemotherapy treatment and was limited to the administration of *Cuscuta campestris* tincture. Treatment was instituted 1 month after bladder tumor removal.

The extracts were obtained from *Cuscuta campestris* Yunck harvested from *Medicago sativa* L. - flowering aerial part (group H1), unselected seeds (group SN2) and selected seeds (group SS2), by extraction at Soxhlet with organic solvents.

The solvents used were: distilled water, ethyl alcohol (20 and 40%), ethyl ether. Some of the filtered extractive solutions were concentrated to the Pfeiffer pump, up to the residue, in the Alpha freeze dryer. Another part of the factions met, before or after the concentration, in different proportions. This resulted in 14 selective extracts, marked A - O, subjected to research, first to be characterized physically and chemically (qualitatively and quantitatively), then clinically.

3. The *chemical analysis* of 20% hydro-alcoholic solution (tincture) of the plant seeds, were *identified*____:βcarotene, β-sitosterol, fitosteroline aglycone (fla terpene and VONICA) flavonosides, phenol-carboxylic acids (AFC), lignans, coumarins, glycorezines, polyholosides.

4. *Quantitative determination* of the active ingredients in the dye gave the following results (expressed in mg% of substance): 71.23–80 flavones (quercetol), 120.07–170.89 phenol-carboxylic acids (caffeic acid); coumarin (7.41–9.16 coumarin), saponozides (foaming index = 100–200 and hemolytic index = 400–500), drying residue = 3–3.20 g%.

In the aqueous solution the same active principles, as in the hydro-alcoholic one, were detected in close quantities.

5. *Testing the antimitotic action* of the infusion and the tincture 20% of the seed plant, on the mitotic film and the root elongation of the seedlings of *Triticum vulgare* Mill. (wheat), showed an antimitotic action of chromatoclastic type.

6. *Acute toxicity (LD 50*) determined by administration to rats of increasing doses, infusion or extract, allows long-term administration; the weight of the animals did not change statistically for samples with $p > 0.05$ (1500 and 4500mg / kg body).

7. *The evaluation of the pharmacological properties* was performed on: the plant cell, rats and a volunteer – a patient with hemorrhagic bladder tumor who was given only 20% turtle tincture, (Cusmed). The treatment was instituted one month after the surgical removal of the tumor – by administration po. Other experiments were performed on people with breast cancer, the treatment being instituted during the break between periods of irradiation with X-rays.

8. *Results*. Cultures of *murine tumor cells EL-4* are inhibited only by the *undiluted* solution, in which the cell suspension is significantly reduced, at 24 and 48 hours, relative to the control and the other extracts. At 48 hours of contact, there are no living cells.

On the *cell ascites 256 Walker* harvested from mice were experienced extract obtained from a mixture of plant species of spontaneous, with solvents of different polarities: chloroform (the extract A1), isobutyl alcohol (extract A2) and ethyl alcohol 50% (A3 extract). The respective extracts were evaporated to dryness in the water bath; the resulting residues were subsequently dispersed in water. The evaluation of the action was carried out after incubation at 37 degrees C.

The anti-proliferative effect observed at 24 hours was slightly different, depending on the solvent. All extracts contain hydrophilic chemical constituents; *o*-dihydroxy-phenols = ODP, flavone saponozide.

The *rats* were observed changes in the cytoskeleton, manifested by *decrease in cell adhesion, due to the retraction protrusion, by affecting actin cytoskeletal F*. The morphological changes are amplified directly in proportion to the contact time. Spontaneously malignant cells are more affected than normal cells (they have cytoplasm almost destroyed by intense vacuolization), especially under the action of aqueous extractive solutions.

Above *melanoma B16* in mice, 50% alcohol extract *has no significant antitumor effects, does not significantly induce survival, but slightly reduces tumor volume*.

9. Dry extract (Cusmed A) and aqueous or hydroalcoholic solution (Cusmed) were tested on a number of volunteers suffering from various forms of cancer. Promising clinical outcomes, in the sense of general condition improvement, partial tumor resorption, prolonged life expectancy, have been reported in some patients with pancreatic head tumor, lower rectal proliferation, bladder tumor, malignant fibrous histocytoma, rhabdo-myosarcoma (RDS) relapsed.

• The *tincture and extracts of Cuscuta campestris did not show cytostatic action* in mice carrying *cells of ascitogenic malignant thymoma EL 4*.

• **Clinical case**: a female person, after a massive hematuria and ultrasound examination, finds out that she is the carrier of a bladder tumor, which is endoscopically resected trans-urethrally. The tumor, brown in color, has dimensions of 1 x 0.9 x 0.6 cm and is subsequently fully processed (for microscopic examination).

The diagnosis, based on the changes of the anatomical tissues that were microscopically observed, was of ***high grade urothelial papillary cancer*** *(WHO / ISUP 2016 classification)* ***histological grade 3 / G3*** *weakly differentiated (WHO classification 1973* ***), invasive in the lamina propria (pT 1 G 3),*** *ICD-O code: 8120/3,* certificate issued on **15-01-2019** by the Emergency Clinical Hospital "Sfântul Ioan", Bucharest.

After discharge, one month after the tumor is removed, the person refuses the classic treatments (BCG vaccine or lectins from *Abies alba*) due to adverse reactions (allergy, inflammation) and mistrust in their efficacy. The mechanism of action is merely an immuno-stimulator. As a result he chooses to treat with the tincture of *Cuscuta campestris* Yunck. He began to daily administer 30 drops of the 1/5 tincture obtained from *Cuscuta campestris* Yunck harvested from the alfalfa (*Medicago sativa*), by maceration in dilute 1: 1 ethyl alcohol (Cusmed - provisional name), for 6 days/week, diluted in 100 ml of water for a long time.

After 2 months after surgery and 1 month of treatment with *Cusmed, the* patient returns to routine control. According to the protocol, a sample for microscopic examination is collected from the scar left after surgery.

Following the *histopathological examination*, the analysis bulletin states: "*fragment of bladder mucosa presenting erosion of the urothelium and rich infiltrated chronic nodular and diffuse inflammatory infiltrate, with gigant-cellular granulomas in the subepithelial fibro-connective tissue (FO 8589, histopathological result no. H6484 -648410 /* ***27.02.2019*** *issued by the University Hospital of Central Military Emergency "Dr. Carol Davila" Pathological Anatomy Service)*".

When he is discharged from the hospital, he states: "it is not necessary to return to the hospital; *no "prescription, medical leave, referral for home care or medical devices" were issued because it was not necessary.*

At the routine check-up, after four months after the surgery and 3 months of treatment with *T-ra Cuscutae,* urethro-cystoscopy is *performed,* after which **there is no recurrence of the bladder tumor** ; from the **old scar; a fragment of bladder mucosa** with dimensions of 0.2 / 0.1 cm **is harvested for microscopic examination**. The certificate issued after the microscopic examination records: "the bladder mucosa fragment presents *erosion of the urothelium and rich chronic inflammatory infiltrate, nodular and diffuse, with gigantocellular granulomas in the subepithelial fibroconjunctive tissue*" (certificate dated **04/04, 2019**).

The patient feels good. She is weak, leads an active life and hopes to maintain her mental balance and her overall health. At the same time, he advises all cancer sufferers not to lose hope in their victory over the disease. Since then, the person concerned administers daily (from Monday to Saturday) 1 teaspoon of *Cuscuta tincture* per day in a glass of water, between morning and lunch. Sunday is a day off.

The efficacy of the treatment is reconfirmed by a new endoscopic exploration of the bladder, performed 6 months after the surgery (on **18. 06. 2019**), by the medical letter stating "*No endovesque tumor recurrences are detected:*

further evolution is favourable". The biochemical analyzes performed after a 4-month cure and the endoscopic control proved the efficiency of Cusmed tincture.

Encouraged by these results, together with my collaborators, we decided to contribute to the dissemination of knowledge of the therapeutic properties of *Cuscuta*, in the hope of extending the life span and increasing the quality of life of bladder cancer patients.

CONCLUSIONS

• The changes observed in the plant and human cell, under the influence of the infusion and tincture of C *secuta campestris* Yunck, confirm the antimitotic action and demonstrate the efficacy in the treatment of bladder cancer.

• We consider that polyphenols – lignin derivatives by cytostatic, antioxidant and permeability maintenance have an important role in the printing of the therapeutic action; carboxylic phenol acids and flavones – by hepatoprotective, capillary-protective, anti-inflammatory, antioxidant, healing and diuretic actions; coumarin by anticoagulant, antioxidant and cytostatic action.

• The influence of the host (***Medicago sativa***) on *Cuscuta'*s metabolism is insignificant, but the contribution of the therapeutic action is in favor of the patient (*Medicago sativa* has a more intense action than *Cuscuta*).

• As a result, we believe that the species *Cuscuta campestris* Yunck can be successfully used in the treatment of bladder cancer, provided that the host plant is not a toxic plant. Research will continue to establish other types of sensitive tumors.

• The chemical researches carried out on *Medicago sativa* L. revealed the presence of phenol-carboxylic acids, flavones, tannins, saponozides and polyholosides. Their amounts in extracts are dependent on the solvent used in the extraction.

• The cytotoxic/cytostatic properties are more intense in *Medicago sativa* L. than in *Cuscuta campestris* Yunck. As a result we consider that for the treatment of cancer, it is not necessary to separate the two plant products.

Nature offers us many living resources, including for the treatment of various diseases. Their use depends on their inspiration, skill and willingness to check and translate them into practice.

Let us join forces, all we who could contribute to the valorizing of the rich *medical folklore* and of the wonderful *natural resources,* less researched.

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