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Autofluorescence of Plant Secretory Cells as Possible Tool for Pharmacy

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Abstract: First experiments for identification of intact plant secretory cells filled with products of secondary metabolism known as drugs based on their fluorescence characteristics has been analyzed on some medicinal plants. Autofluorescence of compounds containing in intact and dried cells induced, mainly, by ultra-violet or violet light was well seen in visible region under various types of luminescent microscopes, including laser-scanning confocal microscope. In the fluorescence spectra of the objects one could see characteristic maxima that may be used in practice of pharmacy. The autofluorescence application gives a possibility to study occurrence and location of natural drugs within tissues and cells. Examples of similar analysis have been considered by use alkaloid-, anthraquinone- and terpenoid-containing medicinal plant species. Especially informative were the fluorescence spectra of pharmaceutically-valuable *Chelidonium majus* and *Frangula alnus* where the emission of alkaloids and anthraquinones, relatively, as drugs prevailed in the natural samples. In first species secretory cells of laticifers contained alkaloids chelerythine and sanguinarine. In more complex, multicomponent samples such as terpenoid-enriched species *Achillea millefolium*, *Artemisia absinthium* and *Calendula officinalis* the interference terpenoids and phenols (brightly fluoresced in glands and secretory hairs) in blue or blue-green emission were observed. Perspectives and difficulties of pharmaceutical analysis with using of autofluorescence were also considered.

Keywords: Anthraquinones, Alkaloids, Fluorescent Drugs, Laser-Scanning Confocal Microscopy, Luminescence Microscopy, Secretory Cells, Sesquiterpene Lactones, Terpenes

1. Introduction

Autofluorescence is a luminescence (induced often by ultra-violet or violet light) of naturally occurring molecules within intact cells or within organism in visible region of the spectra. Fluorescing secondary metabolites of plants that known, mainly, as natural drugs are accumulated in secretory structures [1, 2] and may be of interest for pharmacological analysis of pharmaceuticals in raw materials. The autofluorescence application gives a possibility to study occurrence and location of natural drugs within tissues and cells. It is one of the non-invasive approach without usage of special histochemical dyes. The luminescence in express-analysis, perhaps, permits to know fast is the plant material ready or not for pharmacy. The stage in the development of secretory structures where main valuable drugs are usually concentrated is also a part of the analysis [2]. The metabolism of natural drugs within the structures and their accumulation in the cells and tissues are also observed by the fluorescent method [1-3]. Secretory cells of plants contain various fluorescing phamaceuticals such as alkaloids, terpenes, phenols, flavines, quinones, etc, and their emission differs from chlorophyll as described earlier [1-3]. Basing on the emission characteristics (color and wavelength) scientist could discriminate secretory cells from non-secretory ones as well as identify prevailing component of the secretions [2, 3]. The study of the emission may be of interest for pharmacologists the more so that new book of medicinal plants for pharmacologists [4] does not include the fluorescent method for analysis yet, and bases only on the microscopic images in transmittent light.

The purpose of the paper is to consider perspectives in the autofluorescence application to investigate the occurrence and location of some known natural pharmaceuticals in plant materials.

2. Experimental

2.1. Objects

Objects of research were fresh and dried herbs of greater celandine *Chelidonium majus* L., fam. Papaveraceae, glossy buckthorn *Frangula alnus* Mill (*Rhamnus frangula* L.), fam. Rhamnaceae, common yarrow *Achillea millefolium* L., and common wormwood *Artemisia absinthium* L., and pot marigold *Calendula officinalis* L.fam. Asteraceae.

2.2. Fluorescence Observation

For analysis the intact and dried samples were used.

Materials. Materials for fluorimetry included subject glasses (glass slides), cover glasses, glass cells-cuvettes, for immersion - glycerin, water, air, immersion oil. The fluorescence of the objects was observed with multiplication of objectives x 10, 20, 40 or with water or glycerin immersion x 85, or with immersion oil x 63, 85, 100.

Images of fluorescent cells. Autofluorescence of living cells observed and photographed microscope on the glass slides at room temperature 20-22^{o} as described previously [5] using *Leica* aparatuses (Germany) luminescence microscope Leica DM 6000 B (natural emission colors) and laser-scanning confocal microscope Leica TCS SP-5 (pictures were seen in pseudocolors). As the source of the exciting light in the confocal microscope a mercury lamp-for visual observation and laserArgon/2 (wavelengths 405, 458, 477, 488, 514 nm), HeNe1 (543 nm wavelength) and HeNe2 (633 nm wave length) were used. Registration carried out three channels.

2.3. The Fluorescence Spectra

The fluorescence spectra of were recorded with the above-mentioned laser-scanning confocal microscope Leica TCS SP-5 (Germany). Individual cells or parts of the secretory structures were first identified as a region of interest (shortly ROI). The intensity per cell was determined on integrating the emission from the ROI. Optical probes on the slices were marked by rings or restangles. At quantitative analysis the average error was calculated the four replices for each ROI. The emission intensity determination was also carried out with MSF-2 dual-wavelength microspectrofluorimeter (Institute of Biophysics production, Pushchino) with the special computer program Student *t*-test for studies, measuring the intensity of the emission at two different wavelengths or in one chosen wavelength as a histogram of the normal distribution fluorescent cells (measuring intensity of fluorescence in the blue 460-480 nm, green 520 nm, and red 640-700 nm) [6]. In each experiment on the same slide with the technique counted 100 cells, the data provided on the status bar are statistically as the curve of total distribution of the intensity of fluorescence of cells (the average error of the experience of the four replices, usually 4 glass slides, was done for each case.

Fluorescence spectra of the water and ethanol extracts from the 1 g of studied material were recorded by spectrofluorimeter Perkin Elmer 350 MPF-44B (UK) in 1 or 0.5 cm-cuvettes.

2.4. Reagents

The brand-name drugs (Sigma-Aldrich, United States, Fluka, Switzerland) - artemisinin, o-coumaric acid, sanguinarine, chelerythrine, azulene were used. Absinthin has been isolated and identified according to Lachenmeier [7].

3. Results and Discussion

Among fluorescent drugs prevailing in the plant materials objects (see their images in Fig.1) having alkaloids, anthraquinones and terpenoids have been chosen for analysis. wavelengths by some phenols and terpenoids that fluoresce in blue.

3.1. Alkaloids

As example of alkaloid-enriched species greater celandine *Chelidonium majus* L. (fam.Papaveraceae) was analyzed. This object has segmented laticifers filled with yellowish-brown content. Medical application of the alkaloids is known in practice of cauterization of warts, kondilomb, and at a care of pillomatosis [8-10]. Besides, the herb has many other features: spasmolytic, cholagogue, bitter, alterative, diuretic, laxative, anodyne, purgative, caustic, anti-allergic, anti-inflammatory, abortifacient (applied internally for jaundice, gallstones, and gallbladder disease or externally for eczema, verrucas, and warts as well as in anti-tumor therapy). In the herb and roots of the species alkaloids prevail - up to 2% and 4%, relatively. Here are about 20 alkaloids, such as chelerythrine, sanguinarine, berberine, etc). Figure 1 with color photos represents native fluorescence of stem longitudinal slice of greater celandine *Chelidonium majus* seen in luminescence microscope as the orange fluorescence interior (image a) at excitation by 400-430 nm light. The same picture one could see under laser-scanning confocal microscope in appropriate pseudocolors for the leaf surface (image b) or on optical slice through the leaf tissue (image c) abundant of yellow laticifers. Optical slice of the leaf permits to see yellow-orange laticifer parallel to the veins.

On Fig. 2 (spectrum 1) one can see the fluorescence spectra of laticifer with characteristic main maximum 540 nm with some other 550, 570, 590 peaks as well as small peak 680 nm related to chlorophyll if the object was excited by laser 405 nm. If the excitation was 488 nm the maximum shifted to longer wavelength (spectrum 2). In leaf parenchyma there are no laticifers, and only chlorophyll with maximum 680 nm prevailed (spectrum 3). The comparison of the latex emission with the fluorescence spectra of pure chelerythrine and sanguinarine having equal maxima 590 nm for both compounds demonstrated complex nature of the natural secretory composition of the structure (broken line spectrum of chelerythrine was put on the spectrum 1).

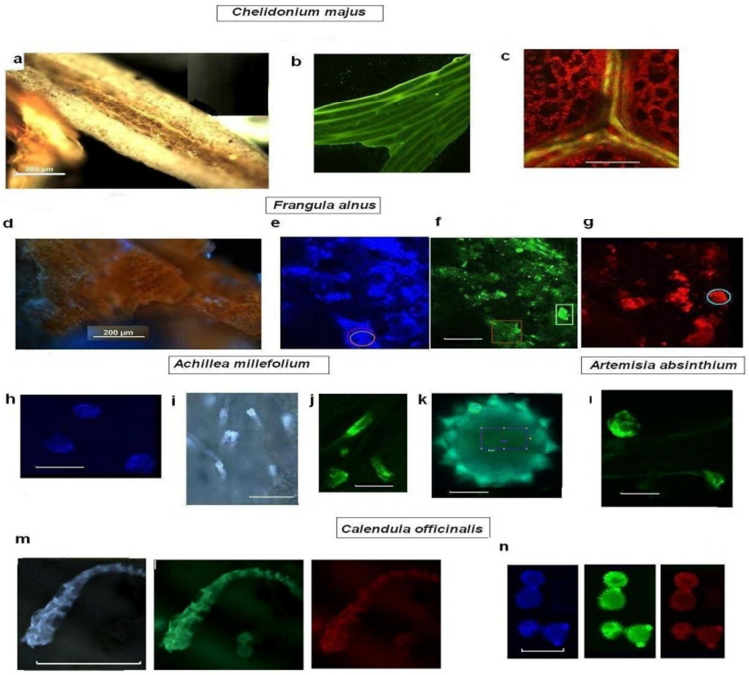

Figure 1. *Views under the fluorescent microscope Leica DM 6000B at excitation 400 nm or 430 nm (a, d, I, m) and laser-scanning confocal microscope Leica TCS SP-5 at excitation with laser 405 nm (b, c, e-h, j, k).*

Chelidonium majus. a – image of longitudinal stem slice, excitation 400-430 nm The brown or yellow-orange laticifers are seen; bar = 200µm; b, c - leaf, excitation laser 405 nm.b- part of external surface of leaf with yellow-fluorescing laticifers (1 channel, bar = 200µm) or c – optical slice (1- 3 channels), bright yellow-orange fluorescing laticifers along the veins are seen to stand out against red background of chlorophyll-containing cells. Bar = 80µm.
Frangula alnus. d – image of bark, excitation 400-430 nm The red-orange cells are seen; bar = 200µm; e-g -
images of bark, excitation by laser 405 nm, registration in 3 channels (blue, green, and red), optical probes on the slices were marked by rings or restangles.
Achillea millefolium. h – image of flower petal with blue-fluorescent glands, excitation by laser 405 nm, bar = 50 µm; i- image of leaf glandular hairs, excitation 400-430 nm, bar = 100 µm; j – image of leaf glandular hairs, excitation by laser 405 nm, bar = 100 µm; k- pollen, excitation by laser 405 nm, bar = 10 µm
Artemisia absinthium. l - – image of leaf gland and glandular hairs, excitation by laser 405 nm, bar = 25 µm;
Calendula officinalis. m – images of secretory hair from dried ligulate flower petal under luminescence microscope when excited by light 340-380,450-490, 515-560 nm, relatively. bar = 25 µm; n- images of pollen in blue, green and red channels under confocal microscope, laser 405 nm, bar = 60 µm.

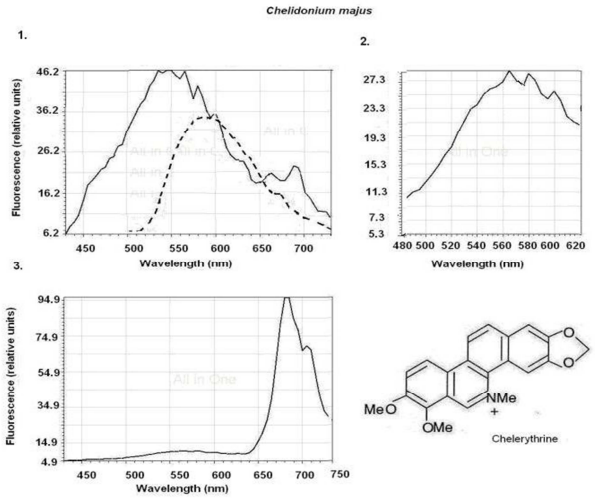

Figure 2. *The fluorescence spectra of alkaloid-containing leaf secretory cells of greater celandine Chelidonium majus recorded by confocal microscope, laser excitation 405 nm. 1. laticifer – unbroken line and crystals of alkaloid chelerythrine – broken line ; 2. laticifer; 3. parenchyma of the leaf.*

Earlier by use of microspectrofluorimetry for the spectral registration the stem and leaf laticifers of celandine have shown to fluoresce in green-yellow with maxima 530-540 nm (excitation 360-380 nm), and in a comparison with peaks of appropriate individual alkaloids (550-580 nm) shift to shorter region is observed [1, 2, 11]. Unlike leaf laticifers, laticifer of flower petal and its latex had maximum 580-590 nm, just corresponding to the maximum of alkaloids berberine, chelerythrine and sanguinarine. Póczi and Böddi [12] studied laticifers and latex of greater celandine laticifers and the native spectral properties of the latex in various organs. Whole plants were studied in a gel documentation system using ultraviolet light source, while the localization of the laticifers was observed along the leaf veins in fluorescence microscope, using blue excitation light. Measuring different tissue pieces, fluorescence spectroscopic studies showed that the greater celandine alkaloids have emission wavelengths at 469, 530-531, 553, 572-575 and 592 nm and excitation bands from 365 to 400 nm. Direct measurements on tissue pieces

(without the extraction and the separation of the components) provided information about the complexity of

the latex and the various ratios of the alkaloid contents in the tissues.These results give a possibility for conclusions about the alkaloid contents and composition or ratios of the alkaloid components in various tissue pieces directly, via comparisons the alkaloids' standards.

3.2. Anthraquinones

Red color anthraquinones are anthracenic derivatives that are found in some medicinal plant species [8-10]. The bark particles from buckthorn *Frangula alnus* Mill. or *Rhamnus frangula Mill*. (fam. Rhamnaceae) known as laxative or bowel tonic relaxants are enriched in oxymethyl anthraquinones such as frangula-emodine, frangulin in A and B-form differencing in the residues, for example gluco-frangulins. This dried bark contains up to 8.5-9.1% of the anthracene-derivative as determined at absorbance wavelength 524 nm [13]. In our work the raw dried material showed red emission in main tissue under luminescence microscope (Fig. 1, image d). Optical probes which have made by confocal microscopy (images e-g) in three different channels – blue, green and red - showed the different location of fluorescing compounds. The complex spectrum with maxima in blue, green and mainly in orange- red (with maxima 590, 600, 620, 640 and 680 nm) in some parts of the bark cells (Fig. 1, images e and f) excited by laser 405 nm was shown (Fig. 3, spectrum 1). Inclusion of chlorophyll (well-seen in red channel of confocal microscope in Fig. 1, image g) may be also seen, especially intensive in maximum 680 nm when excited by laser red light 561 nm (spectrum 2).

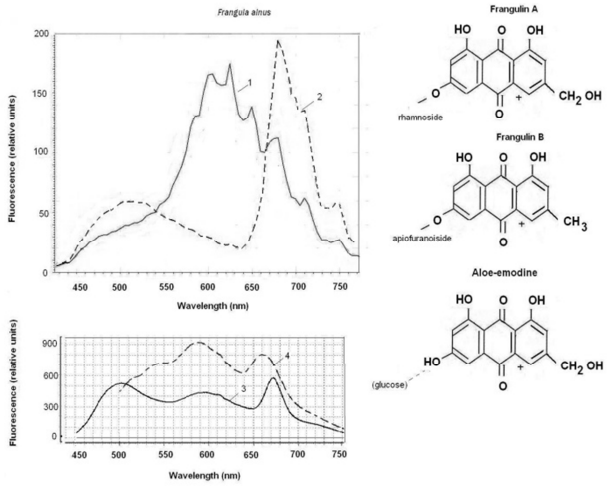

Figure 3. *The fluorescence spectra of bark optical slice from buckthorn Frangula alnus containing anthraquinones frangulins and emodine. 1 and 2 - recorded by laser-scanning confocal microscope Leica TCS SP-5 (laser excitation 405 nm, broken line for the determination of chlorophyll contribution with maximum 680-685 nm in red channel (see g- part of the tissue on Color Fig.1);. 3 -4- the fluorescence spectra of the ethanol extracts from the bark, excitation 405 and 470 nm, relatively.*

The comparison of the fluorescence spectra in situ (from cells) and extracts from 1 g of the dried raw material with 96% ethanol (Fig. 3, spectra 3,4) permits to determine main fluorescence of anthraquinones (emodine, frangulins A and B, etc) between 550-650 nm with maxima about 590 nm.

On the physico-chemical properties of anthracene derivatives such as frangulins A and B as well as fluorophore aloe-emodine are crystalline substances of yellow or orange-red color that fluoresce in red being undergone by ultra-violet light. Anthraquinones frangulines and their derivatives excited by UV-light fluoresce in orange-red with maxima 500, 550 and 650 nm in thin-layer silicagel plates if the spots excited by UV-light [14]. Fluorophore of the frangulins is emodine that lack of the sugar or furan residue and fluoresce with maximum 580 nm and shoulder 548 nm [15].

3.3. Terpenoids

As objects for fluorescent analysis were chosen terpenoid-enriched medicinal species common yarrow *Achillea millefolium* L., common wormwood *Artemisia absinthium* L. and pot marigold *Calendula officinalis* L.

Common yarrow *Achillea millefolium* usually is used in medicine as blood-stopped natural drug at any bleedings and in compositions to care gastritis and inflammation of mucous membranes of gastrointestinal tract. In Fig.1, analyzing of the samples from *A. millefolium*, one can see the blue fluorescent images of glands on the petal (image h) and leaf glandular hairs (images i and j). Fig. 4 shows main emission maximum of the petal glands is 480-500 nm and of leaf hair shifted to shorter wavelength (475-480 nm). Bright blue fluorescence may belong to sesquiterpene lactones prevailed in flower petals and leaves that contain sesquiterpenoids (mainly up to 1% of essential oil that includes about 30% azulenes, geraniol, citral, menthone, carvone, pinene, thujone, cineol, as well as achilleine - alkaloid [9, 10]. Broken line on Fig. 4 shows the fluorescence spectrum of crystallic azulene that emits with maximum 430 nm.

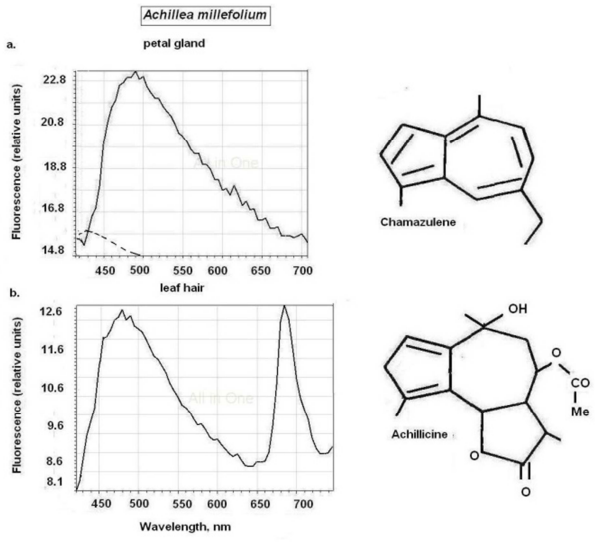

Figure 4. *The fluorescence spectra of gland on the petal surface of flower (a) and glandular hair (b) on lower surface of leaf from common yarrow Achillea millefolium. Laser excitation 405 nm. Broken line is the emission spectra for crystallic azulene.*

According to Murav'eva with co-authors [9] secretory glands

are fulfill of chamazulene (also fluoresce in blue at 420-435 nm) and may be transformed to bitter prochamazulene achillicine. Diploid and tetraploid plants contain proazulene sesquiterpenes, which are transformed to colored azulenes, including chamazulene (up to 25%) and achillicine. Other major constituents in tetraploid plants include α- and β-pinenes (23-28%), as well as caryophyllene (10–22%). Hexaploid plants are azulene sesquiterpene-free. It should mark that unlike leaf secretory cells in petals there is no chlorophyll in the glands, some azulenes bounded with cellulose in cell walls also may give red (maximum 620 nm) emission [2, 16]. It is interesting that pollen of the plant species also fluoresce in blue-green (Fig. 1, image k) with the same maximum 480-500 nm as we saw in vegetative organs (non illustrated). Among the non-essential oil constituents are flavonoids (luteolin and its glycosides, as well as rutin), coumarins and tannins [9]. Perhaps, some flavonoids and coumarins may contribute in blue-green fluorescence [1, 2]. Some phenols and monoterpenes include in the emission (maximum 480-500 nm is peculiar to flavonoids, while emission < 430-390 nm – to monoterpenes), but in smaller degree in the glands and mainly concentrated in secretory hairs [2].

By microspectrofluorimetry it has been demonstrated that composition of the compounds changes during the development of the secretory structures. Only in developing petal glands sesquiterpene lactones such as azulenes prevail as was seen from the maximum 430 nm of the structures, while later new compounds contribute in the glandular emission when the emission maximum shifted to 480-500 nm [2]. Azulenes are also abundant during the developed leaves of white and red clovers *Trifolium repens* and *T. pratense* as well as in chloroplasts of leaves from *Pisum sativum* [17].

Table 1. *The fluorescence intensity of secretory and non-secretory cells on petals and leaves from common yarrow Achillea millefolium. Excitation 360-380 nm (microspectrofluorimety) or 405 nm (confocal microscopy).*

Organ	Wavelength, nm			
	450-460	550	450-460	550
	Microspectrofluorimetry		Confocal microscopy	
Petal of ligulate flower				
Secretory cell (shoulder 460 nm, max. 550 nm)	43±4	25±2	68±3	23±4
Non-secretory cell (max. 460 nm)	10.0±2	7.8±0.4	1.9±0.1	19±2
Leaf				
Secretory cell (shoulder 460 nm, max. 550, 680 nm)	10±0.5	8.3±0.4	12±0.2	10.6±0.3
Non-secretory cell (max. 475, 500, 685 nm)	2±0.3	1.2±0.1	1.9±0.1	1.9±0.1

The emission intensity per a cell was determined by microspectrofluorimetry and confocal microscopy as seen in Table 1. In both cases the secretory cells fluoresced with higher intensity in a comparison with non-secretory cells. Thus, the difference may be used for pharmaceutical practice in the estimation of the mature of the medicinal plant as a whole and, perhaps, enriching in some fluorescent drugs, in particular. In any cases autofluorescence of secretory cells serves as a natural marker when they are seen among non-secretory cells.

Common wormwood *Artemisia absinthium* known earlier as classic stomachic stimulant of appetite today is of the medicinal interest as anticancer drug [10]. In the herb there are 0.5-2% of essential oil [9, 10] that includes weakly or non- fluorescent in UV-region mono- and diterpenes, among which thujone, cadinene, phellandrene, bisabolene. It can also contain anti-cancer drug sesquiterpene lactone artemisinin [18]. If to compare the blue-green fluorescent surface images of the leaf glands and glandular hairs (Fig. 1, image l) with their emission spectra (Fig. 5) one could see that secretions within the structures lightened with maximum 480 (spectrum1 and 2 for both glands and tip of glandular hair), while weakly emitted middle of the hair – with maximum 500 nm (Spectrum 3). Chlorophyll maximum 680 nm practically was not seen or weakly seen in our optical probes. Main fluorescent drugs may be azulenes such as chamazalene, artemazulene and proazulenes with the emission maximum 380- 430 nm [2]. Bitter sesquiterpene lactone absinthin has maximum 550 nm (spectrum 4 measured from crystals of the substance by confocal microscopy). Similar maximum was seen for ethanol solutions of absinthin. Artemisinin and o-coumaric acid emit with peak 500-505 nm (Fig. 5). The contribution of the main components absinthin and azulenes is not seen in the form of certain maxima, although the emission at 550- 620 nm is marked (absinthin fluoresces with maximum 550 nm and better recoded if the excitation was 470 nm). As seen from Fig. 5 fluorescing gland and tip of glandular hair have the emission maximum 480 nm that similar with the maximum of o-coumaric acid. Azulenes in pure form emit in blue at 420-430 nm (see Fig. 4), but is known that they may be accumulated in cell wall [16], and in this case after the binding with cellulose the maximum of the compound was at 620-630 nm.

Among terpene-containing medicinal plants pot marigold *Calendula officinalis* is used due to antibacterial, antiviral and antiinflammatory properties [9]. Their flower baskets and pollen enriched in carotenoids (up to 3% of dry mass) and essencial oils [19]. On Fig.6 and color Fig.1 (images *m*) one can see the fluorescence of the petal surface and individual glandular hair from sepal of ligulate flower. Petal surface includes lightened secretory cavities or ducts seen as the intercellular spaces (possible fulfill with essential oils) and they emitted in blue with maxima 450 nm and small shoulder shorter than 440 nm (Fig.6, images and spectra 1, 2). On the contrast main surface cells weakly emitted in yellow-orange with maximum 540 nm (image and spectrum 3).

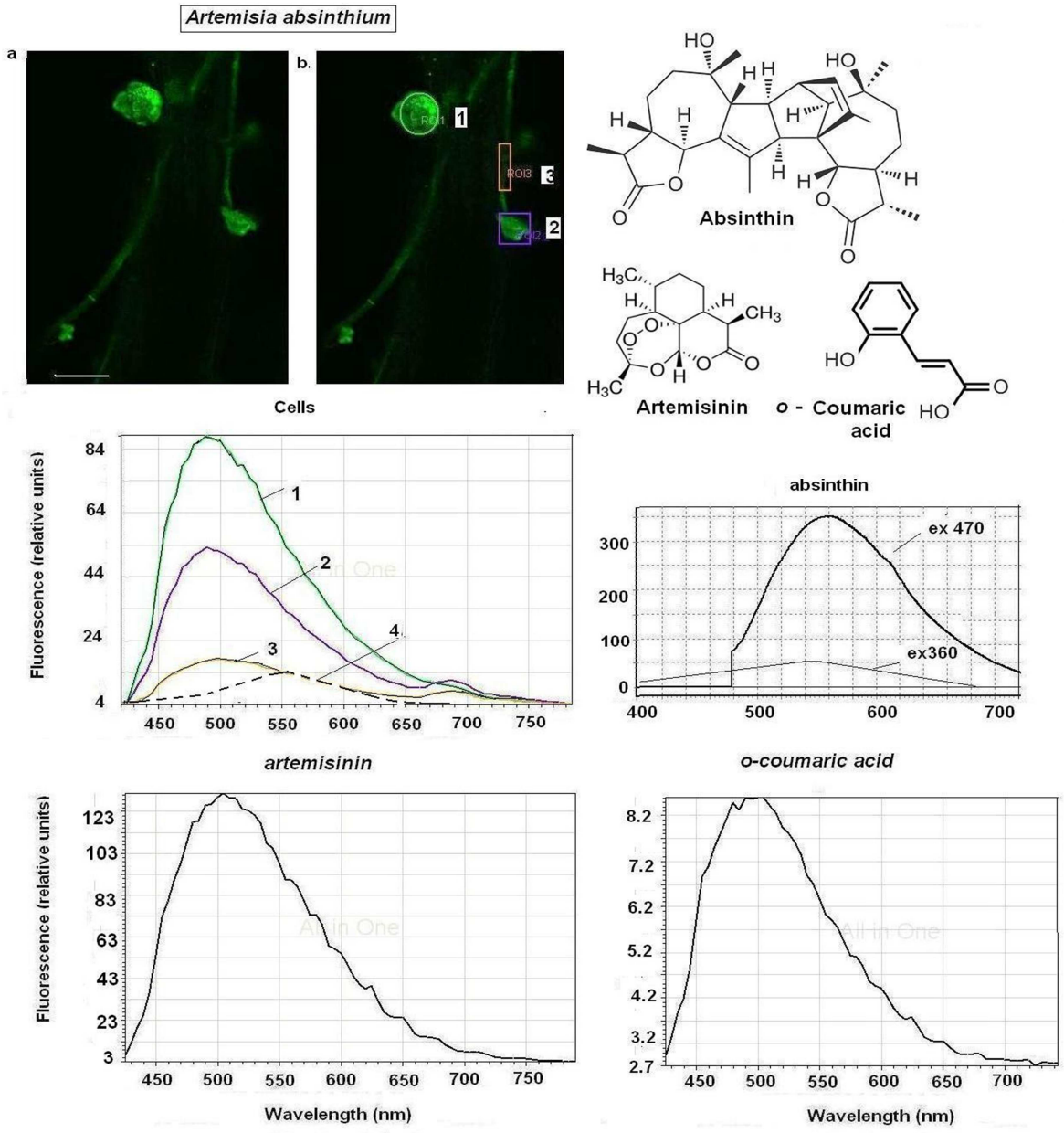

Figure 5. *The fluorescence images (a,b) and spectra of gland (1), secretion in the tip of glandular hair (2) or middle part of the hair (3) on lower surface of leaf from common wormwood Artemisia absinthium (numbers of the measurable parts are on b-image, bar = 25 μm) and crystallic absinthin (4), characteristic compound for the species. Laser excitation 405 nm. The fluorescence spectra of the ethanol solution of absinthin (right) were recorded by Fluorimeter Perkin –Elmer at the excitation 360 nm and 470 nm. The fluorescence spectra of pure individual drugs of the herb such as crystallic artemisinin and o-coumaric acid were recorded by laser-scanning confocal microscope (laser excitation 405 nm).*

It is similar with known peak for the β-carotene crystals fluoresced with peaks 520-534 nm or - with shoulder 500 nm and peak 590 nm in concentrated ethanol solutions [2]. Unlike based petal cells sepal glandular hair emitted in blue, green and red. In confocal microscope we could saw the difference in the emission of capitate top cells of the intact multicellular hair. One of them fluoresced mainly in yellow (Fig. 6, image and spectrum 1) while the second -in blue-green spectral region with maximum 475 nm (Fig. 6, image and spectrum 2). In the cells of stalk chloroplasts were seen, and chlorophyll brightly emitted in red with maximum 680 nm (Fig. 6, image and spectrum 3). When the trichome in dried herb was analyzed under usual luminescence microscope the blue, green an red emission is seen at excitation 340-380, 450-490 and 515-560 nm, relatively (Fig. 1, images m). Moreover, the structure looks as biseriate multicellular hair. In blue flavonoids, coumarins and quinones may fluoresce in petals, while tannins and other polyphenols of cell wall weakly fluoresce in blue and green. Pollen of the species also fluoresces, and middle part of the pollen grain has maximum 530-540 nm, similar with petal.

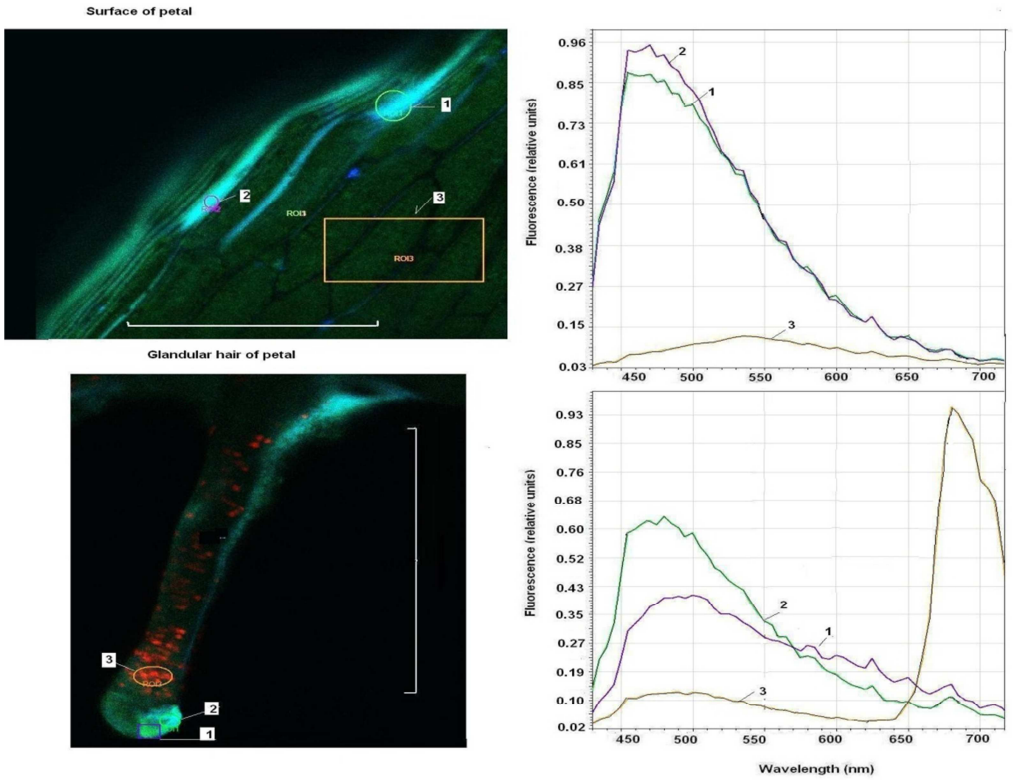

Figure 6. *The fluorescence images and spectra of Calendula officinalis at excitation by laser 405 nm, Upper side – sum in thee channels of ligulate flower petal, bar = 50 μm; Lower side – of the sepal secretory hair from the same flower, sum in blue, green and red channels, bar = 60 μm. Numbers on the images and spectra show concrete ROI – optical probe.*

This peak is peculiar to β-carotene [2]. Apertures, from which pollen tube grows, emits brighter with the same maximum or 520 nm. The petals and pollen of *Calendula officinalis* contain up to 3% of dry mass of carotenoids such as β-carotene and its oxidized derivatives violoxanthin, flavoxanthin and auroxanthin, etc [9] that may contribute to the fluorescence. Bright green emission also, perhaps, belonged to β-carotene (abundant in the orange petals) [2].

3.4. Perspectives and Difficulties

Along to the visible preferences in the fluorescent analysis of intact pharmaceutical material we need to show limitation and additional factors influencing on the determinations as well. Some above-mentioned examples of the autofluorescence that was observed in secretory structures of medicinal plants are only first steps in the application of the parameter to express-analysis in pharmacology without damage of intact tissues. Similar results as in living cells were fixed in dried material too, although with higher intensity because the presence of water in the material may quench the emission completely or partly. The influence of stress, ageing or pest invasion may be also take into consideration when one analyze the fluorescence of secretory products [1, 2, 20]. For example, defensive compounds such as phenols may be formed and accumulated in the tissues at certain doses of ozone in the air [5].

It also needs to keep in mind the difficulties and problems in the identification of the compounds in the samples based on the autofluorescence. First of all it is interference of the wavelengths by some phenols and terpenoids that fluoresce in blue. It is overcome by the extraction of the components by hydrophilic and hydrophobic solvents like it is described earlier and then measurement of the fluorescence spectra [2]. The disappearance or shift of maxima in the fluorescence spectra after similar procedures shows that some compounds extracted, for example, by hydrophilic solvents, contributed in the emission of intact cells, like earlier was seen for pollens [2]. In complex mixture of natural composition masking of the weak fluorescence, peculiar to one component, by bright emission of another component in the same spectral region may take place too. In this case by the extraction of the masking components by hydrophilic or hydrophobic solvents is also recommended, and then it should compare the fluorescence spectra of the samples before and after the appropriate extraction procedure.

Differences in the position of maxima one and same plant species appear to depend on the media of secretory cells, which may additionally contain oil, phenols, oleophenols, oleoresins and others in combinations found in the cells [21]. Here it is likely to use modeling between main component analyzed and possible medium by a preparation of mixtures between compound analyzed and medium. After that one could record their fluorescence spectra in order to determine true maximum and shifts [2, 22]. For example, crystals of

phenol rutin emit with maximum 620 nm, but in mixture with menthol oil - 480-485 nm, while crystals of alkaloid rutacridone with main maximum 595-600 nm stored the same peak in similar mixture [2]. All this should be subjects of attention in future investigations of application of the fluorescent characteristics of secretory cells of medicinal plants.

4. Conclusion

One of the non-invasive applications for pharmacy is the luminescence observation of plant materials - tissues and cells of some medicinal plants. Being excited ultra-violet or violet light most pharmaceutical-containing secretory structures (individual cells of bark, secretory hairs and glands of leaves and flowers) studied fluoresce that permit to discriminate them among non-secretory cells impoverished in natural drugs. Without histochemical dyes in express-luminescent analysis it appears to know quality and readiness of the samples using the emission of the secretory cells where pharmaceuticals are accumulated. The estimation of secretory cells basing on their autofluorescence as a natural drug marker has perspectives in the pharmtechnology. Using the fluorescence spectra of secretory cells the identification of prevailing pharmaceuticals (for example, alkaloids and anthraquinones) according their characteristic maxima is also possible. For multicomponent-fluorescing secretory cells such as in terpenoid and phenol-enriched species belonging to family Asteraceae the identification of the individual compounds based on their autofluorescence spectra, perhaps, needs additional procedures.

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