Formulation and Preparation of Pharmaceuticals with Anti-rheumatic Effect Using the Active Principles of *Capsicum Annuum* and *Piper Nigrum*

OLIMPIA DUMITRIU BUZIA[1], NELA MARDARE[1], ALEXANDRA FLOREA[2], CAMELIA DIACONU[1], RODICA MIHAELA DINICA[3]*, ALIN LAURENTIU TATU[1]

[1]Dunarea de Jos University of Galati, Faculty of Medicine and Pharmacy, Unit Research Center in the field of medical and pharmaceutical sciences Dunarea de Jos, Pharmacology Sciences Department, Romania, Al. I. Cuza Str. 35, 800017, Galati, Romania

[2]College of Pharmacists Braila, Faltis SRL, 27 Galati Str., 810388, Galati, Romania

[3]Dunarea de Jos University of Galati, Faculty of Science and Environment, 111 Domneasca Str., 800201, Galati, Romania

The present paper aims at obtaining two pharmaceutical formulas based on two active principles extracted from two plants well-known for their contents of capsaicin and piperine, respectively, Capsicum Annuum and Piper Nigrum. After the extraction of the active principles, we pursued their identification using chromatographic and spectrophotometric methods. Subsequently, we produced two pharmaceutical formulas, an ointment and a cream, in view of using them in rheumatic diseases as anti-inflammatory medicine.

Keywords: capsaicin, piperine, ointment, cream

Plants have been considered important sources of pharmaceutical compounds since the Antiquity, civilization being inherently connected with the plants' world. For millennia, they have been a major source of obtaining bio-products essential for the survival of the entire animal regnum. The healing effect of the plants has been acknowledged and experienced since the old days. The anti-inflammatory effect of the active principles in plants [1] has been successfully used in various pharmaceutical formulas, from solutions, stupes, ointments or creams, for the treatment of rheumatic diseases [2]. Rheumatic diseases are among the major causes of work inability and determine the increase in costs of medical care. They significantly reduce the quality of life because of their specific symptoms – pain, articular ankylosis, decrease in the strength of the affected joints, etc. [3].

Experimental part

Materials and methods

We used dried fruits of *Piper Nigrum* and hot pepper, harvested in mid-August, and then dried. *Capsicum annuum* is a widespread species of *Capsicum* genus, *Solanaceae* family. The dried hot peppers, especially the ones used in the production of paprika, after the removal of seeds, have the following average chemical composition [4]: water 10.8%, azotic substances 15.86%, extractive substances and nitrogen 24%, fats 12.5%, cellulose 20.9% and ashes 6.8%.

The dried hot peppers are richer in certain vitamins, containing 100-300 mg/100g Vitamin C, 22-23 mg/100g pro-vitamin A (fig. 1).

It has been noted that raw hot pepper contains seven times more vitamin C than a lemon [5]. The largest quantity of vitamin C is found in the ground red pepper - 10 kg of pepper contain 369 mg of vitamin C, compared to only 47 g in lemon. Energetic value: 21 Kcal/100g. 200 g of hot pepper provide the necessary of vitamin C for a day for an adult.

Hot pepper is a digestive stimulant, vitaminizing, revulsive, recommended in digestive asthenia and atone dyspepsia. Researchers from Nottingham University revealed that the active principle of the hot pepper is a substance called capsaicin [6]. Hot pepper has been proven a natural analgesic and, what is more, having anesthetic effects, as researchers from Harvard Medical School of Boston, Massachusetts used capsaicin in combination with another substance for this latter purpose. The presence of luteolin has also been underlined. It acts over the memorizing processes, inhibiting innate or acquired behaviors. This substance is able to block the damaging effect of certain noxae on the nerve cells (neurotoxins) and to determine the activation of the neural circuits involved in the memorizing and learning processes. Lutein also helps in maintaining the health of the eyes. Pepper also contains lycopene (a red-colored substance with antitumor effects in prostate, breast, ovary, cervical, pancreatic, lung and skin cancers). It represents a natural source of vitamin C and beta-carotene. It has antioxidant effects, strengthening the immunity system; it prevents the diseases produced by free radicals: cancers, heart diseases. It also has hypocaloric effects and can be used in diets [7]. An experiment conducted at Cedars-Sinai Medical Center of Los Angeles has showed that injections with capsaicin (the substance which gives the taste of hot pepper) may induce the self-destruction of cancer cells up to 80% and may determine the remission of tumors in prostate cancer. Capsaicin is an antioxidant which protects the cell DNA, its anticancer action being also proven for breast, ovarian and lung cancers. Owing to capsaicin, hot pepper may also prevent cerebral strokes and may reduce the cholesterol levels.

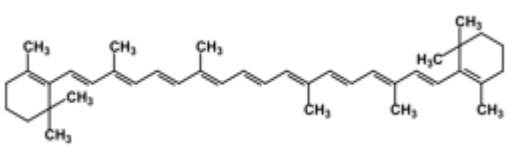

Fig.1. beta-carotene

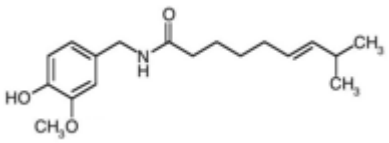

Fig. 2. Capsaicin

* email: rodica.dinica@gmail.ro

All authors have had equal contribution to designing and writing the presented paper.

Capsaicin is a substance found in *Capsicum annuum* (hot pepper), responsible for the extreme hot sensation experienced at the contact with the oral mucosa during ingestion. Although its effects have been noted since the old times, it is only now that it is well-deservedly included among the substances of great healing potential.

Piperine (fig. 3), the second extract, is well-known for its antioxidant and anti-inflammatory properties.

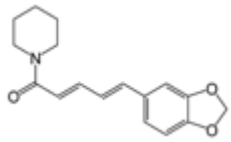

Fig.3. Piperine

Piperine is the piperidine of piperic acid. Piperic acid is formed by hydrolysis, next to piperidine, from the piperine alkaloid, one of the components with pungent taste of pepper [8]. Isopiperic acid, a stereoisomer of piperic acid, has been obtained from piperonal by condensation with acetaldehyde, followed by Perkin condensation with acetic anhydride and sodium acetate. Piperine was discovered in 1819 by Hans Christian Ørsted, who isolated it from fruits of *Piper Nigrum* (black pepper).

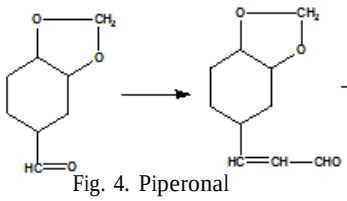

Fig. 4. Piperonal

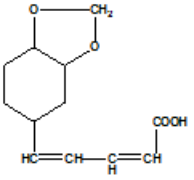

Fig.5. Isopiperic acid

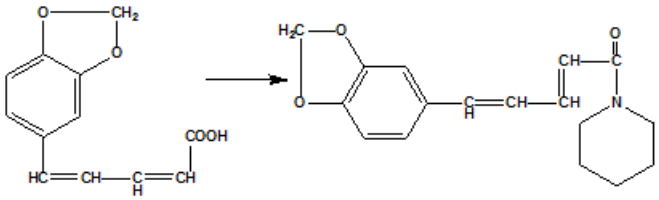

Fig. 6. Piperine

Results and discussions

We used the method of simplified extraction of the active principles with alcohol, using ultrasonic bath followed by filtration and evaporation with the rotary evaporator. We extracted the two active principles, namely capsaicin and piperine. Then, we used thin layer chromatography and spectrophotometry in view of highlighting the active principles and other compounds of the extracts.

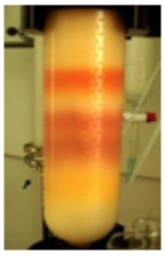

Fig.7. Column Chromatography - capsaicin

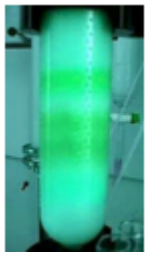

Fig.8. Column Chromatography -piperine

The hot pepper extract contains 0.06% capsaicin, and the pepper extract contains 1.2% piperine.

For recording the UV-Vis spectra, we used the UV-Vis double beam PC spectrophotometer. The solutions obtained were analyzed following dilution, as the initial solutions had a 10% concentration.

The obtained spectra are presented in the figures below.

Following spectrophotometry, the conclusions below could be drawn:

In figure 9-the UV-Vis spectrum of the alcoholic solution obtained from dried hot pepper- one notes the presence of maximum characteristics owing to the functional groups

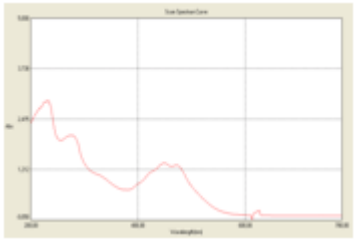

Fig.9- UV-Vis Spectrum obtained from dried *Capsicum annuum* (hot pepper)

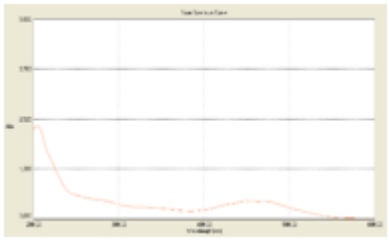

Fig.10- UV-Vis Spectrum obtained from fresh hot pepper

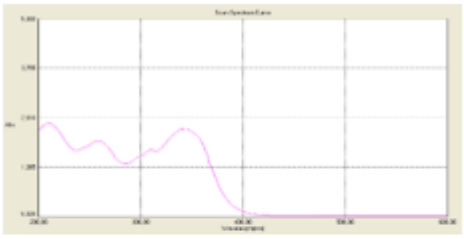

Fig.11-UV-Vis spectrum obtained from *Piper Nigrum* (black pepper)

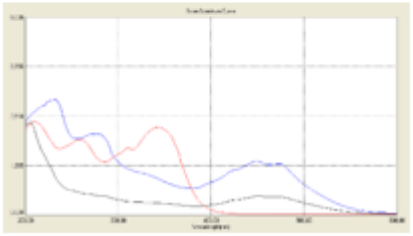

Fig.12-Overlapped spectra

in the extract. Thus, one notes a maximum level in the 450 nm (VIS) area, which is specific to beta carotene. Also, in the 260-290 nm area, one notes a maximum specific to compounds with aromatic nucleus, such as flavonoids or polyphenols. In figure 10 - the UV-Vis spectrum of the alcoholic solution obtained from fresh hot pepper, one notes that the maximums are much lower, which means that bioactive compounds are found in a significantly less amount in this extract compared to the extract obtained from dried hot pepper. The presence of beta-carotene was observed at c. nm.

The UV-Vis spectrum of the *Piper Nigrum* extract, in figure 11, proves the presence of bioactive compounds, such as piperine, which present three specific maximum levels at ~ 230 nm, ~250 nm, and at ~370 nm.

By overlapping the three spectra, as shown in figure 12 (Overlapped spectra)-one can assert that the two spectra obtained from *Capsicum annuum* (hot pepper) contain the same compounds in different proportions, whereas the extract obtained from *Piper Nigrum* (black pepper) presents other chemical compounds.

Therefore, using UV-Vis spectrophotometry, we could emphasize the relation between the concentration of the substance analyzed and absorption.

The next step was to produce two pharmaceutical forms in the laboratory of pharmaceutical technology: an ointment and a cream containing the isolates, together with other active substances with anti-inflammatory and analgesic properties, such as: methyl salicylic, camphor, aetheroleum eucalipti, etc. Epidermal ointments act at the skin level, having low penetration strength and producing local effects. They are primarily recommended in protecting the skin against the irritating effects caused by external agents. It is believed that such ointments enter

the epidermis only superficially. As excipients for the preparation of such ointments, hydrocarbons are especially recommended - vaseline, silicones, A/U emulsion bases or polyethylene glycols. Endodermic ointments act at the skin level, penetrating its layers and releasing medicament solution [9]. The ointments should not modify the skin *p*H, as it has an acid *p*H (4.5-6.5), which ensures certain protection against bacteria [10]. For the preparation of the ointment used for the amelioration of rheumatic pains, we used the following formulas:

Table 1
FORMULATION OF THE OINTMENT AND CREAM

		Ointment	Cream
Active substance	U/m	Quantity	Quantity
Methylii salicilas	gram	5	5
Tinctura capsici	gram	5	5
Tinctura piperinum	gram	5	5
Camphorae	gram	4	4
Oleum helianthi	gram	10	
Adeps lanae	grame	20	
Vaseline	gram	20	
Aetheroleum eucalypti	drops	-	30
Alcoholum cetilicum	gram	-	7.5
Glycerol	gram	-	7.5
Natrii lauril sulfas	Gram	-	0.5
Aquae distillata	gram	-	35

In order to test the effectiveness of the two ointments, we conducted a study on a 10-people sample, aged 35 to 75, who suffer from joint pains.

In choosing the people who were to test the two ointments, we considered the following aspects: the subjects are not allergic to one of the compounds, they suffer from joint pains, and they are willing to abide by the recommendation they receive. Each person received 30g of ointment. Five of them received an ointment sample, and other five, a cream sample. They were asked to apply the ointment in a thin layer, on the painful area, slowly massaging until the full absorption into the skin. In order to increase the ointment's effectiveness, after its being applied on skin, the painful area should be kept warm.

Conclusions

We can conclude that, following the extractions from the two plants, namely *Capsicum annuum* and *Piper Nigrum*, we obtained the active principles, namely capsaicin and, respectively, piperine. Both the hot pepper and black pepper extracts contain bioactive compounds. After chromatography, we noted that the extract from hot pepper contains 0.06% capsaicin, and the extract from black pepper contains 1.2% piperine.

We produced two pharmaceutical forms with anti-inflammatory and analgesic action. The patients who used the ointment had a lower satisfaction degree compared to the patients who used the cream, as the latter, owing to the properties of the excipients, allowed the in-depth treatment of the joints pains. There is a possibility to associate the external treatment with systemic products with delayed action, using modern pharmaceutical formulas capable to slowly release the active principles [11, 12, and 13].

Also, starting from the observation of the antibacterial and anti-inflammatory properties, with regard to medical ethics and capitalizing the patients' informed consent [14, 15], further studies could be pursued on the effectiveness at the cutaneous level in selected cases in which certain medicine cannot be administered, being counter-indicated because of tachyphylaxis, unexpected adverse reactions, intolerance, lack of response, or unexpected phenomena also described in relation to anti-inflammatory medication or medication prescribed for different comorbidities [16-26]

Observations can be extended in the future towards the approach of in vivo/in vitro inhibition of endosymbionts, alterations of the microbiome, in dysbiosis [27-33]. The adjuvant role of the treatment in various infections can also be played in the future by these active principles, at first, at the experimental level, and then in vivo [34, 35]. Another promising indication is related to the usage of *Piper Nigrum* extract in the treatment of vitiligo, a cutaneous disease which causes color losses, occurring either by itself or associated with other autoimmune diseases, where the requirement of immunosuppressing, anti-inflammatory treatment could be complemented with natural extracts [36-38]. Experimentally, in the future, the natural extracts, either by themselves or combined, acting synergistically, could become useful in the prophylaxis of areas described as *locus minoris resistentiae*, or in the Wolf isomorphic phenomenon, which entails the occurrence of another dermatosis, different from the initial one, at the spot of cutaneous healing, a phenomenon which can also be understood as Koebner phenomenon type 5 [39-41]. Another possible domain can be represented by its use for ameliorating zoster or post-zoster pains, where capsaicin has a clearly quantified role owing to its capacity of inhibiting the P substance involved in the mechanism of zoster pain or that of other neuropathic pains or paresthesia.

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