

Curcumin- Extraction, Physical and Chemical Analysis, Formulas and Control. Basic Methods for Further Research

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Turmeric, with its active component curcumin has been regarded lately as an important potential therapeutic agent due to its properties and many uses. Further research needs to be done both on animals and humans in order for it to be used at a large scale. As curcumin gets absorbed better through topical and not oral administration, curcumin-based pharmaceuticals with skin passage must be devised. In order for this to be done, the need for a standardized, verified and simple extraction method and one for ointment preparation with stability in time rises. We propose in this study a method for curcumin extraction, one that ensures an adequate stability in time and a method for pharmaceutical control.

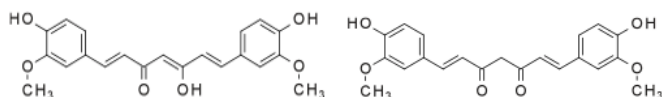
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Since ancient times people have studied nature and plants inherently concerning their healing effect on the human body. Plants have been considered indisputable curative means -natural remedies for humans and animals.

Researchers have sought after their bio-properties, bioavailability, possible interactions, adverse effects, their pharmacological uses or their efficacy and safe use both in animals or human subjects.

Turmeric is one of the many plants with a multitude of beneficial health -properties which nature makes available for people. Besides its use as a condiment or pigment, turmeric, a source of curcumin, has been used with medicinal purpose in India (*Curcuma longa*) for centuries. Proof that curcumin has anti-inflammatory and anticarcinogenic activity has renewed the scientific interest concerning disease prevention and treatment.

Curcumin is the main natural polyphenol found in the rhizome of *Curcuma longa* (turmeric), having antioxidant, anti-inflammatory, antimutagenic, antimicrobial, antiparasitic and anticancerous properties.



Enol form

Keto form

Administered orally, curcumin has low bioavailability due to the fact that it is poorly absorbed by the gastrointestinal system. As a topic, this polyphenol is better absorbed and it is such used in various skin diseases -inflammation and lesions.

The wide number of diseases in which curcumin acts as a therapeutic agent is a direct result from its various pharmacological properties: antioxidative, anti-inflammatory, anticarcinogenic, antimutagenic, antiproliferative, anticoagulant, antiviral, antibacterial, antifungal, antiprotozoal, antidiabetic, hypotensive, hypocholesterolemic, UV protective, wound healing, choleric, antifertility, antifibrotic, antiulcerative or antivenomous[1-8]. One needs to emphasize the fact that synthetic drugs (such as tetracycline, hydrochlorothiazide, statins, NSAIDS, beta blockers or topical steroids) have systemic and cutaneous toxic pharmacological side effects and that secondary Staphylococcal or other types of infections can occur on the skin (with unnecessary cutaneous microbioma or incipient lesion changes) or in any other organ[9-37].

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The main objective of our research was to obtain curcumin-based pharmaceuticals. Concerning the pharmaceutical manufacturing, we extracted curcumin from *Curcuma longa*, made the physical and chemical analysis of the extracted formula and finally realized the quality control of the pharmaceutical form.

Experimental part

Materials and methods

When extracting the active compounds from various animal or vegetable products with the help of a solvent, with or without the following step of concentrating the extracted compound (liquid, soft or solid states), the procedure must take notice of the nature of the drug to be extracted, of the active compounds that it contains, the chemical structure of the active principles that determine the solubility in various solvents and its heating stability.

The chosen method of extraction is the continuous one which takes place with the help of the Soxhlet apparatus composed from a balloon, an extraction body and an ascendant refrigerant, all of them linked together. This method was chosen due to the following advantages that it presents itself with: the lack of necessity for filtration, a lower quantity of solvent used (lower than in maceration) and it is a continuous process.

The selected solvent chosen for the component extraction from turmeric is introduced in the balloon and it is placed in a water bath. The extractor is attached over the balloon with the help of a rubber plug sealed off with collodion. A cartridge with the solid substance is imbedded in the extractor. This cartridge is made up from filter paper and the substance gets triturated before being introduced in it. Before introducing it in the extractor, the cartridge is weighed with the help of the analytical balance. The position of the cartridge needs to be precise so as it will not surpass the level of the siphon. The refrigerant is mounted over the extractor and the connection to the water source is made. The solvent is heated and its vapors get to the refrigerant - here they cool off and condense; from here they flow out in the extractor, over the cartridge. The condensation level rises and as it does, the intended extraction of the compound from the solid takes place. When the liquid from the extractor reaches the superior side of the siphon, this starts and the solvent with the extracted component get transferred into the balloon. By continuing the heating process, new vapors of solvent reach the refrigerant - they follow the process and new quantities

of extracted component are taken over by the solvent. Again, when the level of the siphon is reached, the liquid from the extractor goes in the balloon. After every cycle of extraction the solvent is enriched with the extracted component. 10 to 15 cycles later, the presence of the extracted component needs to be verified in the solvent from the extractor. If the component to be extracted is no more, the heating is stopped, the installation gets to cool off (the water circuit functions during this step of the procedure), the cartridge is taken out, it is dried and weighed. The difference between the initial mass and the final mass of the cartridge represents the quantity of substance extracted from the solid mass (the component). By knowing the quantity of substance introduced in the cartridge, the percent of the component extracted from the solid material can be calculated. The extraction is considered to have been done in total, having 8 to 9 h duration.

The spectrophotometric analysis done by absorption is used to identify the substance, its purity degree and the titration, based upon the property of the substance to selectively absorb electromagnetic radiations. The materials and apparatuses used for this technique are: ethanol, ethanol extract, test tubes, quartz dishes, Spekol 11 spectrophotometer and pure curcumin and they were used as such: the apparatus was standardized, the samples were introduced in the quartz dishes and these in their special base and the absorbance was registered at the necessary wavelengths.

Three variants of ointments were formulated: a lipophilic one, a hydrophilic one and one emulsion-type A/U, in which we added our extracted solution (0.16g%) as stated in table 1.

Table 1
OINTMENT PREPARATION

Components	Ointment 1	Ointment 2	Ointment 3
Lanolin	1g	7.83g	-
Vaseline	9g	3g	-
Cetaceum	-	2.95g	-
Cholesterol	-	1.46g	-
Olive oil	-	9.76g	-
Alcohol extract	0.08g	0.08g	0.08g
Corn starch	-	-	3.5g
Nipagin	-	-	0.09g
Nipasol	-	-	0.01g
Glycerin	-	-	46.5g
Distilled water	-	25g	3.5g

The quality control of the ointments was done - their homogeneity, color, smell, pH determination (with the help of the pH-meter), adhesion capacity (Ojeda -Arbussa method), penetrating determination (in a Berzelius glass was an amount of ointment and from a fixed height a glass measuring baguette fell -with known dimensions and weight; the length of the portion that penetrated the mass was noted). When stability was determined we kept the samples at cold, hot and room temperatures, in the light and in the dark with the help of a lid box in which 5g of sample are introduced and are held in the aforementioned conditions for 15 and 30 days [38-42].

Results and discussions

The extraction of curcumin from turmeric powder (*Curcuma longa*) was done with the help of the materials and the exact steps of the extraction technique described earlier. After the extraction (9 h duration), from 10 g of turmeric powder and 150 milliliters of ethanol, 17 milliliters

of alcoholic extract were obtained. The solution was clear, orange, and it smelled like curcumin and ethanol; it was used for spectrophotometric analysis and ointment preparation.

First it was necessary to obtain the standardized scale from 10mg of curcumin dissolved in 100 mL of ethanol (100 µg/mL) from which 0.1-0.5 mL were pipetted and diluted up until 10mL with ethanol (1-5µg/mL). The spectrophotometric analysis was done by measuring the absorbance of this substance at 422 nm wavelength. Table 2 presents the results of this analysis, along with figure 1.

Concentration (µg/ml)	Absorbance (at 422 nm)
1	0.160
2	0.324
3	0.483
4	0.651
5	0.831

Table2
CONCENTRATION AND ABSORBANCE OF THE SOLUTION SAMPLES FOR STANDARDIZED CURCUMIN

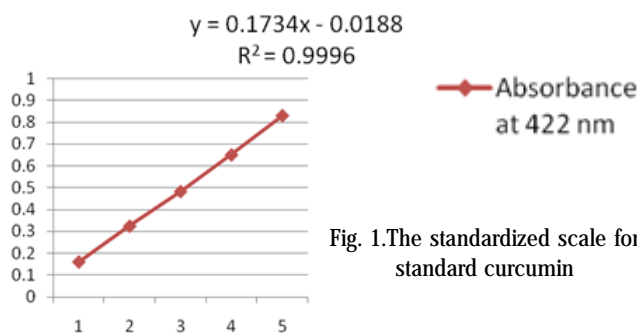


Fig. 1. The standardized scale for standard curcumin

The equation of the direct line obtained in the standardized scale for standard curcumin is used in order to evaluate the curcumin concentration in the analyzed samples:

$$y = 0.1734x - 0.0188$$

$$x = \frac{y + 0.0188}{0.1734}$$

$$y = \text{absorbance}$$

$$x = \text{standard curcumin concentration}$$

0.1 mL of the extract was introduced into the balloon of 100mL of ethanol (it was used as a witness). The absorbance was measured at 422 nm wavelength with the help of the Spekol 11 spectrophotometer.

$$y = 0.215$$

$$y = \text{read absorbance}$$

$$y = 0.1734x - 0.0188$$

$$x = \frac{0.215 + 0.0188}{0.1734} \Rightarrow x = 1.348 \mu\text{g}$$

So from 0.1 mL extract we obtained 1.348 µg and from 17 mL, 229.16 µg curcumin. 100 g of turmeric powder has 2291.6 µg of curcumin, meaning 2.2916 mg% curcumin. 10 g of turmeric has 0.22 g of curcumin and 100 g has 2.2 g curcumin.

Concerning the 3 ointments (presented in fig. 2) prepared in line with the technique described earlier, the results were as following: Ointment 1 featured a homogenous appearance and a semisolid consistency with a yellow-orange color and a pH of 6. The second one was also homogenous and semisolid, orange in color, with a pH of 6.5 and the third ointment was a translucent, hygroscopic, hydrophilic gel with a pH of 7. All of them had the

characteristic smell due to their components and were also compatible with the pH of the skin.



Fig. 2. Ointment 1, 2 and 3 as prepared in the laboratory.

By using the Ojeda-Arbussa method, the ointments' capacity for adhesion/spreading was evaluated (table 3.)

Table 3

OINTMENTS 1, 2 AND 3 - ADHESION (SPREADING) CAPACITY (OJEDA-ARBUSSA METHOD)

	Ointment 1	Ointment 2	Ointment 3
First plaque	5 cm	5.3 cm	6.7 cm
100 g	5.5 cm	5.7 cm	7.5 cm
200 g	5.6 cm	6 cm	7.8 cm
300 g	6 cm	6.2 cm	8.2 cm
400 g	6.4 cm	6.5 cm	8.2 cm
500 g	6.5 cm	6.7 cm	-

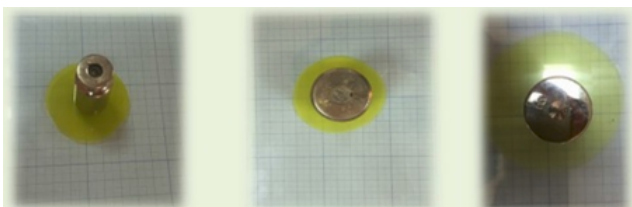


Fig. 3. Ointments 1, 2 and 3 - adhesion (spreading) capacity (Ojeda-Arbussa method)

The results are pointing towards the fact that the adhesion/spreading capacity rises as the weights being added increase. As the surfaces become larger, the ointments have a higher capacity for adhesion/spreading. Data analysis reveals that Ointment 1 has a lesser capacity for adhesion/spreading than Ointments 2 and 3; this is in direct link with their initial consistency. By adding the 500 g weight, Ointment 3 spread/adhered no more due to the fact that it is a gel.

The penetration of the ointment (meaning the degree of its consistency) was also evaluated and it established that the length of the product's penetration into the product's mass was 5 cm (from a maximum length of 10 cm).

The stability of the 3 products was analyzed at 15 and 30 days from preparation and the characteristics evaluated were aspect, color and smell. Heat stability was unchanged for the 3 ointments at 15 days, but at 30 days it was modified for all. Cold stability was not modified for the products both at 15 and at 30 days. Stability to light was modified for all after 15 and 30 days, while dark stability was not changed in all cases. Room temperature stability was not changed for all ointments at 15 and 30 days. Thus curcumin is characterized as a photosensitive substance, being easily degraded by light.

The pH of the three ointments is shown in table 4.

Ointment	pH
1	6
2	6.5
3	7
Extract	3

Table 4
pH OF THE PREPARED
PHARMACEUTICALS

All of the experimental preparations have a pH compatible to that of the skin.

Conclusions

We have obtained a preparation that contains curcumin. Three ointments from the lipophilic and hydrophilic categories have been formulated. After turmeric extraction, an ethanol extract of curcumin was obtained, quantitatively titrated by the spectrophotometric method and obtaining a concentration of 2.2g% of curcumin.

After organoleptic analyses of the 3 ointments, the following observations were made: they have a characteristic aspect of the components and a specific smell. Their pH was in between the interval imposed by the Romanian Farmacopeea 10th Edition. By verifying their stability we concluded that they are cold-stable, dark-stable and room temperature-stable. These will be kept in tightly sealed recipients, at room temperature, safe from light and with a validity term of 3 to 5 months.

The therapeutic potential of curcumin is immense and there is data to support this statement. It may also be of interest to further research the prophylactic or healing effects on pigmented skin conditions, cutaneous and nevus photo protection (in order to prevent the Koebner phenomena), cancer or autoimmune disorders, as well as the possible use in pregnancy (having the patients' informed consent) both for curcumin and for other natural extracts (indigo naturalis for example). [43-59] In order for this to be brought to light further studies must be done in the future with the help of our method for obtaining curcumin-based ointment.

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