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Prospects of Using Freons as Extractants of *Curcuma Longa* L. Root Essential Oil

Elena Zhilyakova
Pharmaceutical technology department
Belgorod State National Research University
Belgorod, Russia
EZhilyakova@bsu.edu.ru

Oleg Novikov
Shared research and educational center
Peoples' Friendship University of Russia
Moscow, Russia

Dmitriy Pisarev
Shared research and educational center
Peoples' Friendship University of Russia
Moscow, Russia

Nikolay Boyko
P*harmaceutical technology department,*
Belgorod State National Research University
Belgorod, Russia

Konstantin Nikitin
Pharmaceutical technology department
Belgorod *State National Research University*
Belgorod, Russia

***Abstract*—the purpose of the present study was a comparative study of the ability of some freons to extract the essential oil of the roots of *C. longa* L. and to provide optimal conditions for its analysis. The dried, crushed roots of *C. longa* L. were used as raw materials (country of origin - Uganda). Methoxynonafluorobutane (*Novec* 7100), fluoroketone and n-hexane were used as extractants. Method of gas-liquid chromatography with mass detection was used. The most efficient extractant for ar-tumerone and curlone, which proved to be methoxynonafluorobutane (Novec 7100). The results of the studies indicate the prospects of using freons as extracting agents for the extraction of essential oil from the roots of *C. longa* L. The prospect consists in a more effective extracting ability of organofluorine solvents than n-hexane to increase the yield of both, the essential oil itself and the most pharmacologically relevant root components of *C. longa* L. - ar-tumerone and curlone.**

Keywords*—*C. longa L. roots, ar-tumerone, curlone, methoxynonafluorobutane, fluoroketone, n-hexane.

I. INTRODUCTION

Curcuma longa L. plant of the ginger family – Zingiberaceae, native to Southeast Asia, South America, India, and is widely used as a spice and medicinal agent in traditional medicine of the countries in this area [1].

The plant is a valuable medicinal and food crop, due to its chemical composition, primarily essential oil and curcuminoids. Due to its unique chemical composition, *C.longa* L. has a number of important pharmacological properties. In particular, anti-inflammatory, anti-allergic, antioxidant, hypoglycemic and antitumor properties of the plant were found [2]. Some components of the plant, in particular ar-tumerone and bisacuron, have a hepatoprotective effect against alcoholic liver lesions [3]. Rhizome essential oil, from different habitats, exhibits antimicrobial activity of varying intensity. Some oils significantly reduce the severity of inflammatory cytokines, cyclooxygenase-2 (COX-2) and tumor necrosis factor (TNF)- α in vivo [4].

Currently, on the basis of *C. longa* L. drugs with antitumor activity are developed and drugs with choleretic, anti-inflammatory, antimicrobial properties, as well as drugs for the treatment of neurodegenerative and other pathological conditions of the brain are produced [2-4].

The chemical composition of *C. longa* L. depends on the harvesting sites of the plant. First of all, the component composition of the essential oil of the plant varies. *C. longa* L. essential oil studied by gas chromatography-mass spectrometry showed the presence of more than 75 components. The main components were ß-tumeron, ar-tumerone, epi-α-patchulene, ß-sesquifellandren, 1,4-dimethyl-2-isobutylbenzene, (±) -dihydro-ar-tumerone, zingberin, atlanton and (-) - oxide caryophyllin [5].

In the sample of essential oil obtained by liquid extraction under pressure (PLE) and subsequent analysis by gas chromatography-mass spectrometry was discovered dominant components, including α-caryophyllin, ar-curcumen, zingiberin, β-bisabolene, β-sesquifellandren, ar-tumerone [6, 7]. The eighty-one components were identified in the essential oils of Chinese samples of rhizomes of C. longa L. obtained by hydrodistillation and analyzed by chromatography-mass spectrometry. The yield of essential oils varied from 4.03 to 5.27% depending on the habitat. The main compounds were ar-tumerone, β-tumeron, α-zingiberen, ar-curcumene and β-sesquifelland [2].

The second significant group of *C. longa* L. compounds are curcuminoids, which include mainly 3 components: curcumin, desmethoxycurcumin and bisdemethoxycurcumin [8, 9, 10]. The structure, composition and quantitative evaluation of curcuminoids in roots and various plant compositions containing curcuminoids was carried out by different methods, namely nuclear magnetic resonance spectroscopy, high-performance liquid chromatography with ultraviolet and mass detection, circular chromatography [11-18].

The method of infrared spectroscopy with Fourier transform (FTIR -spectroscopy) was used to quantify the content of the curcuminoids – curcumin and demethoxycurcumin in the ethanol extract of the roots of Javanese turmeric – C. xanthorrhiza Roxb. HPLC with photometric detector (HPLC/PDA) was used as a comparison method.

A simple method for estimating curcumin content in multicomponent plant compositions by UV spectrophotometry [15] as part of dietary supplements by high-performance thin-layer chromatography (HPTLC) [9] has been developed.

The method of ultra-high performance liquid chromatography / mass spectrometry (UPLC/UVMS) is proposed to determine the curcuminoids and ar-cumarone in the roots of *C. longa* L. and different species of Curcuma (*C. zedoaria*, *C. phaecaulis*, *C. wenyujin* and *C. kwangsiensis*), and dietary supplements containing *C. longa*.

The study of this plant is carried out in line with the development of a new scientific direction "pharmaceutical remake" [19].

Essential oil of *C. longa* L. roots is one of the active groups of biologically active substances of the plant, responsible for a unique diverse pharmacological action. Therefore, the search for appropriate conditions for the production and analysis of this group of substances is an urgent task.

The purpose of the present study was a comparative study of the ability of some freons to extract the essential oil of the roots of *C. longa* L. and to provide optimal conditions for its analysis.

II. EXPERIMENTAL

Freons – methoxynonafluorobutane (Novec 7100) and fluoro ketone were used to select the optimal extractant capable of providing the maximum yield of active components. N-hexane was taken as an extractant of comparison. The choice of these freons as extractants of *C. longa* L. essential oil is due to several reasons:

- the low boiling point (60 °C) of these freons determines the gentle temperature mode of extraction and their easy removal from the extracted amount of biologically active substances in the preparation of a pure essential oil complex. This, in turn, eliminates the processes of destruction and isomerization of individual components of the phytocomplex, as well as additional energy costs;
- the viscosity of these freons is immeasurably lower than that of traditional organic solvents, which determines their extraction properties with a higher diffusion potential;
- chemical indifference defines such properties of these refrigerants as non-inflammability and explosion, which allows to work safely with these extractants;
- positive ecological profile and toxic safety profile, namely absence of ozone destruction and global warming potentials, as well as complete absence of toxicity to living organisms [20].

The dried, crushed roots of *C. longa* L. were used as raw materials (country of origin - Uganda). To extract the essential oil from the roots of C. longa L. 1.0 g (exact weight) of the raw material, 10 ml of the appropriate extractant was poured, tightly capped and extracted by maceration at room temperature during the day. After this time, the resulting extraction was filtered through a paper filter "blue ribbon". The obtained extracts were directly used for chromatography.

As an optimal variant of chromatography, we have chosen the method of gas-liquid chromatography with mass detection (chromatography-mass spectrometry, GS-MS), which allows us to establish the saturated composition of the essential oil of C. longa L. without using standard samples as witnesses and simultaneously determine their content.

Chromatography was performed on the device chromato-mass spectrometer model GCMS-QP2010 Ultra, manufacturer "Shimadzu", Japan. The ion source of the mass spectrometer is carried out in the electronic shock mode. Ion separation is carried out by a quadrupole mass filter, detection by a secondary electron multiplier with a reversed dynode. Detection was performed by total ion current (SCAN). Since the essential oil composition of the roots of *C. longa* L. is represented by mono -, sesquiterpenes and aromatic derivatives having different vapor elasticity, chromatography was performed in the mode of programmable temperatures.

The separation was carried out on the column:

1. Zebron ZB-5MS 30 m L × 0,25 mm ID × 0,25 μm df;
2. Liquid phase: 5%-polysilarylene-95polydimethylsiloxane;
3. Temperature limits:- 60C° to 325/350°C;
4. Carrier gas - helium with constant flow-3.0 ml / min;
5. Column temperature-100° C (isotherm 2 min) - 230° C (isotherm 5 min), the rate of temperature rise 5 deg / min;
6. Evaporator temperature-250°C;
7. The temperature of the ion source-250°C;
8. Interface temperature-250°C;
9. Mode input sample without dividing the flow time – 2.5 minutes;
10. Voltage detector – 0,88 sq;
11. The flow of emissions – 60 μA;
12. Sample input volume – 1μl.

Detection was carried out in the mode of total ion current (SCAN) in the range m/z 30 – 500 Da, with a scanning speed of 1000 and a resultant time of 0.5 seconds. Identification of the components was carried out by comparing the mass spectra with those available in the electronic library NIST 11.

III. RESULTS AND DISCUSSION

The chromatogram of the separation of the essential oil from the roots of *C. longa* L. after extraction with methoxynonafluorobutane is shown in figure 1.

The composition of extraction from *C. longa* L. extracted with the use of synonafluorobutane labels has 34 components, 8 of which are the main ones, ar-tumerone and curlon are dominant.

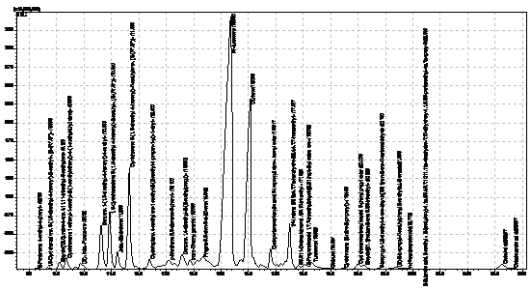

Fig.1. Chromatogram of separation of essential oil components of *C. longa* L. roots extracted with methoxynonafluorobutane

Chromatogram of separation of components of *C. longa* L. roots, after extraction fluoroketone is shown in figure 2.

The fluoroketone extraction component composition included 25 components, 8 of which dominate, the major ones are ar-tumerone and curlone. The chromatogram of separation of hexane extraction from the roots of *C. longa* L. is shown in figure 3.

The hexane extract contains 39 components, among which is dominated by ar-tumerone and curlone.

Comparative evaluation of the extractive capacity of the selected solvents was carried out according to the criteria-logarithm of the peak area of the component on the chromatogram - the content of the component in the sum (%) on the corresponding chromatogram. The logarithmic function of the peak area of the corresponding components was chosen because of the cumbersome values of the peak areas on chromatograms.

A summary table of the comparative extracting capacity of the selected extractants for the extraction of essential oil from the roots of *C. longa* L. is presented in Table I

Thus, the results presented in Table I show that the typical components present in all three extracts are nine of which the largest amount of contained ar-tumerone and curlone All presented compounds belong to the class of sesquiterpenes and their aromatic derivatives.

After analyzing the results given in Table I, it can be concluded that methoxynonafluorobutane extracts the components in the largest quantities.

Fluoroketone is inferior in extraction efficiency to the indicated solvent, and it extracts the complex of biologically active compounds of turmeric n-hexane in the least amount (Fig.4.). The scheme of the extracting ability is given in relative units, that is, relative to the most effective extractant, the total area of the logarithms of all peaks of which is the largest.

Concerning the extracting ability of the selected extractants to isolate the most important pharmacologically and quantitatively dominant components of ar-tumerone and curlone, the following results were obtained (Fig.5.).

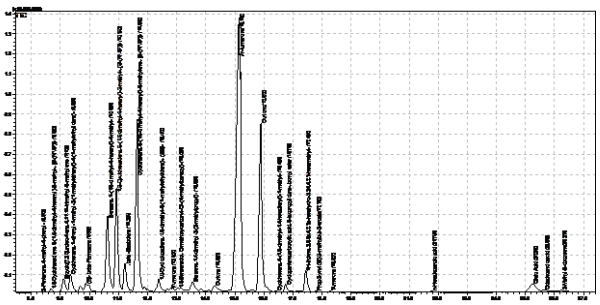

Fig.2. Chromatogram of separation of components from the roots of *C. longa* L., extracted with fluoroketone.

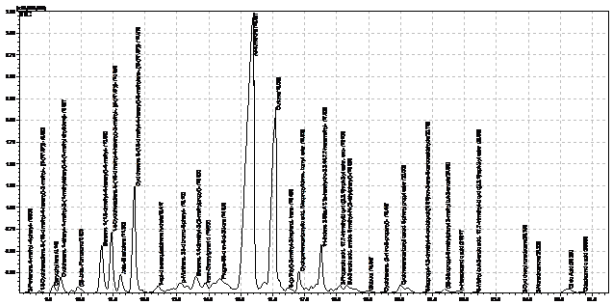

Fig.3. Chromatogram of components separation from *C. longa* L. roots extracted with hexane

TABLE I. SUMMARY TABLE OF THE COMPARATIVE ABILITY OF EXTRACTANTS TO EXTRACT ESSENTIAL OIL FROM THE ROOTS OF *C. LONGA L.*

№ peak	Component holding time, min	Logarithm of peak area (lnS), after extraction by selected extractants: *Extractant*			Component content in the sum, % on the chromatogram obtained after extraction with selected solvents			Component name, formula
		Novec	*Fluoroketone*	*Hexane*	*Novec*	*Fluoroketone*	*Hexane*	
1.	10.6	17.7	17.2	17.6	3.57	7.03	3.49	Alpha curcumin
2.	10.9	17.8	17.33	17.76	3.96	8.00	3.83	Alpha zingibern
3.	11.2	16.75	16.22	16.75	1.37	2.64	1.40	Beta-bisabolen
4.	11.6	18.39	17.89	18.36	7.08	14.06	6.99	Beta sesquifellandren
5.	12.403	16.51	15.62	-	1.08	1.45	-	Germacren B
6.	14.3	17.71	-	-	3.57	-	-	Alpha bergamoten
7.	15.3	20.14	18.82	20.09	40.57	35.76	39.36	Ar-tumerone
8.	16.0	19.38	17.95	19.3	18.91	14.87	17.93	Curlone

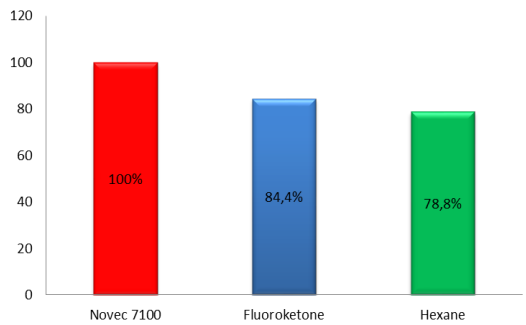

Fig.4. Diagram of freon extraction ability to extract essential oil from *C. longa* L. roots.

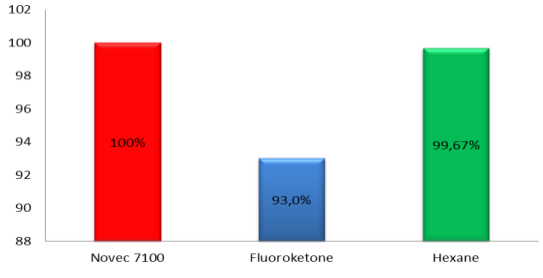

Fig.5. Diagram of the extracting ability of freons to remove ar-tumerone and curlone.

Diagram of the extracting ability of the solvents is also given in relative units, that is relative to the most efficient extractant for ar-tumerone and curlone, which proved to be methoxynonafluorobutane (Novec 7100).

IV. CONCLUSION

The results of the studies indicate the prospects of using freons as extracting agents for the extraction of essential oil from the roots of *C. longa* L. The prospect consists in a more effective extracting ability of organofluorine solvents than n-hexane to increase the yield of both, the essential oil itself and the most pharmacologically relevant root components of *C. longa* L. - ar-tumerone and curlone. In addition, these extractants are devoid of toxicity, environmental, fire and explosion. In addition, the optimal conditions for chromatography of *C. longa* L. essential oil were selected, consisting in the use of chromatography mass spectrometry in the mode of programmable temperatures in the range of 100-230 °C with a constant flow rate of carrier gas 3 ml / min without flow division, interface temperature and evaporation of 250 °C.

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