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Testing Phenol Compounds in Spices

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Abstract: Cardamom fruits and carnation buds were tested by high-yield liquid chromatography (HPLC) method. 12 phenol compounds were found in cardamom fruits: gallic acid, isoferulic acid, chlorogenic acid, epicatechin, chicory acid, caffeic acid, dehydroquercetin, ferulic acid, luteolin, quercetin, rutin, o-methoxycoumarin, cinnamic acid, in carnation buds - 12 phenolic compounds: gallic acid, catechin, isoferulic acid, chicory acid, coffee acid, dihydroquercetin, ferulic acid, luteolin, quercetin, rutin, 0- methoxycoumarin, cinnamic acid. The methods of internal normalization show that gallic acid is prevailing compound in cardamom fruits and gallic acid and quercetin- in carnation buds. Quantity of amount of flavonoids in terms of rutin in carnation buds was measured by differential spectrophotometry. It varies from 3.55 % to 5.10%. Tanning agents in carnation buds and cardamom fruits were measured by permanganate titration, they are respectively 4.11- 5.02 % and 0.02- 0.06 %.

Key words: Cardamom % Clove tree % Phenol compounds

INTRODUCTION

Treatment with phytogenous preparations becomes more and more popular now both in Russian and world practice. Research of phytogenous bioactive substances including food plants as perspective sources of vegetable crude drug is the question of pressing importance.

Elettaria cardamomum (L.) Maton is perennial plant of gingery family with large rhizome that forms several herb stems up to 2-3 m high. Now the plant is being cultivated in numerous tropical countries-India, Thailand, Guatemala, on Ceylon and Malay Archipelago. Seeds of ripe fruits extracted right before preparation are used. Cardamom fruits are officinal preparation in many countries [1, 2, 3].

Syzygium aromaticum (L.) err. et Perry is evergreen tree of myrtle family with coriaceous opposite smoothed-edge and small purple-pinkish flowers assembled in clusters. Now this plant is being cultivated in many tropical countries. Buds are used – dried flower buds that are officinal preparations in many countries [1, 4, 5].

Analysis of bibliographic data proves that chemical compound of essential oil of raw cardamom [6-11] and clove tree [12-18] is researched to relatively high extend.

The purpose of the work is testing phenol compounds and measuring this group of natural compound in cardamom fruits (*Elettaria cardamomum* (L.) Maton) and carnation buds (*Syzygium aromaticum* (L.) err. et P) as perspective sources of vegetable crude drug.

Methodic. Industrial series of cardamom fruits conforming requirements of GOST 29052-91 “Spices. Cardamom. Technical requirements” and carnation buds conforming requirements GOST 29047-91 “Spices. Carnation. Technical requirements”.

High-yield liquid chromatography (HPLC) by GILSTON, model 305 (France), small injector RHEODYNE 7125 (USA) was used to research qualitative evaluation of phenol compounds of cardamom and carnation raw materials. Research results were processed by software MultiChom for Windows. Metal column with 4,6×250 mm form-factor Kromasil C 18, sorbent particles size 5 mkm was used as fixed phase, the system methanol- ware-concentrated phosphoric acid (400:600:5)- as moving phase. Analysis was carried out in room temperature with eluent supply speed 1 ml/min. Analysis duration was 70 min. Ultraviolet detector GILSTON UV/VIS 151 model was used for detection with 254 nanometers wavelength.

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Analyzed sample was reduced to fragments of such size that allows passing through the sieve with openings diameter 2 mm. About 2.5 g of raw material was introduced into retort with the volume 100 ml added 20 ml 70-percent ethyl alcohol. It was attached to reflux condenser and heated in boiling water bath for 2 hours from the moment of simmer of alcohol solution in retort. After cooling the solution was filtered through paper filter in volumetric flask with volume 25 ml. The volume of the substance was extended by 70-percent ethyl alcohol to the mark (analyzed solution). Simultaneously a series of 0.05-percent reference solutions of phenol carbonic acids, flavonoids and coumarin in 70-percent ethyl alcohol were prepared.

Than 50 mcl of analyzed solutions and reference solutions were introduced in chromatograph and were chromatographed according abovementioned method.

Separated substances were identified by comparing time of solution compound retention with time of reference compound retention on the base of not less than 5 chromatograms. Measuring of phenol substances in analyzed samples was made by peak areas by internal normalization method.

Quantitative evaluation of flavonoids in carnation buds was made by differential spectrophotometry after complexing reaction with aluminum chloride. Terms of flavonoids extraction from raw material: granulation, leach and extraction time were analyzed during development of this method. Analysis of UV absorption spectrum of spirit extraction of carnation buds shows that maximum extraction was observed with wavelength 408 nanometers, the same maximum had 0.05-percent rutin solution after complexing reaction with aluminum chloride. So 402 nanometers was taken as analytical wavelength and quantitative measurement of amount of flavonoids in terms of rutin in 3 replications was made with this wavelength.

Bud sample for analysis was reduced to fragments of such size that allows passing through the sieve with openings diameter 1 mm. About 1.0 g (exactly) of granular buds was introduced into 250 ml slice, added 50 ml 40-percent ethyl alcohol. The retort was weighed with error $\pm$ 0,01, that it was attached to reflux condenser and heated in boiling water bath for 2 hours. Then retort was cooled to room temperature and weighed. If necessary, 40-percent ethyl alcohol was added to restore initial mass. Retort content was filtered through paper fold filter, first 25 ml of filtrate was separated (solution A). In 25 ml volumetric flask 1 ml of solution A was added, 3 ml of 2-percent alcoholic solution of aluminum chloride was added and the volume was restored to the mark with 96-percent ethyl alcohol. Solution consisting of 1 ml of solution A and 0.1 ml concentrated acetic acid and restored to the mark with 96-percent ethyl alcohol was used as reference solution. After 40 min optical density of tested solution was measured with spectrophotometer with 408 nanometers wavelength in a pan with 10 mm thick stratum.

Content of amount of flavonoids in perfectly dry raw material in terms of rutin in percent (X) was calculated according the formula:

$$X = \frac{A \cdot 50 \cdot 25 \cdot 100}{248 \cdot a \cdot 1 \cdot (100-W)},$$

where:

A- optical density of solution;

248- specific value of rutin complex with aluminum chloride absorption for the wavelength 408 nanometers;

a- raw material mass in gr;

W- raw material humidity in percent.

Tanning agent content was measured by permanganate titration method according to recommendations of State pharmacopeia, XI edition in 3 replications [19].

Main Body: The research allows finding 12 phenol compounds in cardamom fruits (Table 1). Gallic acid is the main compound of phenol compounds that was determined by the method of internal normalization. 12 phenol compounds were found in carnation buds (Table 1). Gallic acid and quercetin are the main compounds of phenol compounds as determined by the method of internal normalization.

Analysis of industrial volumes of carnation buds by method of differential spectrophotometry shown that content of amount of flavonoids in terms of rutin varies from 3.55 % to 5.10 %.

Developed methods were validated on linearity, repeatability, laboratory and correctness. Linearity was tested on 5 levels of concentrations from theoretical content of amount of flavonoids in carnation raw materials. Correlation coefficient shouldn't be lower that 0.99. In the experiment it was 0.997. Repeatability was tested on one sample of raw material in 6 replications.

Table 1: Phenol compound in cardamom fruits and carnation buds

	Cardamom fruits		Carnation buds	
Compound	Retention time, min	Quantity of compound in mixture, %	Retention time, min	Quantity of compound in mixture, %
gallic acid	2.94	30.47	3.24	15.80
catechine	-	-	3.93	8.53
isoferulic acid	4.54	3.25	4.22	21.85
chlorogenic acid	4.96	1.22	-	-
epicatechine	5.78	2.11	-	-
chicory acid	6.27	2.47	6.32	1.42
caffeic acid	7.07	2.27	6.71	3.59
dehydroquercetin	11.26	0.66	11.69	1.00
ferulic acid	13.67	2.41	13.45	4.08
luteolin	15.34	8.19	15.28	1.78
quercetin	18.38	2.41	18.52	13.63
rutin	22.15	3.04	23.39	3.00
o-methoxycoumarin	-	-	32.21	0.45
cinnamic acid	52.16	0.06	52.26	1.16

Acceptability criterion was expressed by the measure of relative standard deviation that shouldn't be higher than 10%. In the experiment it was 1.49 %. Reproducibility was tested by two chemists who used 3 samples in three replications. Acceptability criterion was expressed by the measure of relative standard deviation that shouldn't be higher than 15 %. In the experiment it was 3.77 %. Correctness of the methods was proved by measuring qualitative content of amount of flavonoids in solutions by adding necessary amount of standard rutin sample to analyzed solution. Average percentage of recovery corrected for 100% was accepted as acceptability criteria and the average measure should be (100$\pm$5)%. It was shown that it varied in the limits of 98.31 % and 104.62 % with the average 100.99 %.

Qualitative content of tanning agent per tanning for carnation buds was 4.11 – 5.02 %, for cardamom fruits 0.02 -0.06 %.

CONCLUSION

Research shows that cardamom fruits and carnation buds have the complex of phenol compounds in their content. Phenol complex includes flavonoids, phenol carbon acid, coumarin and tanning agents. By method of high-yield liquid chromatography, (HPLC) content was analyzed. 12 phenol compounds were found in cardamom fruits and 12 phenol compounds in carnation buds. Gallic acid is the main compound of phenol compounds in cardamom fruits and gallic acid and quercetin- in carnation buds that was determined by the method of internal normalization.

Quantity of amounts of flavoniods in terms of in carnation buds is 3.55 – 5.10 % and tanning agents- 4.11 -5.02 %. In cardamom fruits there are 0.02- 0.04 tanning agents.

Resume. Phenol compound of cardamom fruits and carnation buds are interesting for further research and using in domestic medical practice as the sources of valuable bioactive substances.

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