Research Paper

Study on the Influence of Origin and Vinegar Roasting Process on the Chemical Composition of Nutgrass Galingale Rhizome

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Cao *et al.*: Study on the Influence of Origin and Vinegar Roasting Process of Nutgrass Galingale Rhizome

To provide a basis for the development of quality standards by studying the changes in the chemical composition of nutgrass galingale rhizome of different origins, before and after its vinegar roasting and to provide a basis for the correct evaluation of the quality changes of nutgrass galingale rhizome of different origins, before and after its vinegar roasting. A thin-layer method was developed to identify the water-soluble components of aconite. The volatile oil content of nutgrass galingale rhizome was determined according to the volatile oil determination method in Appendix XVI, Part I of the 2010 Chinese Pharmacopoeia. The volatile oil content of nutgrass galingale rhizome from Shandong and Anhui was higher (1.59 % and 1.61 %), while the volatile oil content of nutgrass galingale rhizome from Guangdong was the lowest (1.14 %). The highest content of beta-sitosterol was found in Anhui (0.017923 %) and the lowest in Guangdong (0.010893 %). The total flavonoid content of nutgrass galingale rhizome from Henan was the highest (0.95381 %) and the total flavonoid content of nutgrass galingale rhizome from Shandong was the lowest (0.63565 %). The volatile oil content and beta-sitosterol content decreased and the total flavonoid content increased in all three groups after vinegar roasting. Shandong and Anhui Nutgrass Galingale Rhizome have better medicinal efficacy and the use of vinegar roasting method for the concoction of nutgrass galingale rhizome is reasonable.

Key words: Nutgrass galingale rhizome, vinegar roasting, volatile oil, beta-sitosterol, total flavonoids

Chinese medicine is a valuable treasure accumulated by the Chinese people during their long struggle against disease. Its rich knowledge and effective practice contain profound scientific connotations and it has gradually formed its own unique theoretical system during thousands of years of medical practice, which has been protecting the health of the Chinese nation for 5000 y and is an important part of the outstanding culture of the Chinese nation. At present, the annual sales of botanical medicines in the international market is about 16 billion United States (US) dollars and its growth rate has far exceeded compared to that of chemical drugs. Modernization of Chinese medicine is an inevitable trend in the development of Chinese medicine and a necessity to embark on the international market. The quality control and management of Chinese medicine is the key to its modernization. Chinese medicine is a traditional national industry in China. With the accelerated development of the modernization of Chinese medicine, a series of problems related to the quality of Chinese medicine need to be solved urgently. If the quality of Chinese herbal medicines is not well controlled, the quality of Chinese medicine preparations will not be able to meet the requirements, the clinical effect will be poor and the safety of people's lives will be greatly endangered, therefore the quality control of Chinese herbal medicines is one of the key to the modernization of Chinese medicine.

The quality control of Chinese medicines includes the identification and content determination of Chinese herbal medicines and proprietary Chinese medicines. The current quality standards of Chinese medicines are very imperfect and confusing and even counterfeit Chinese medicines are often seen in the market, seriously endangering the safety of people's medicines. In order to control the spread of counterfeit and substandard herbs, it has become a consensus that research on the quality of Chinese medicines must be strengthened and a better, more accurate and authoritative method of evaluating the quality of Chinese medicines must be developed. The quality of Chinese herbal medicines is mainly based on the difference in the content and type of chemical

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components they contain.

Nutgrass galingale rhizome is the dried rhizome of *Salvia divinorum*. It is also known as *Cyperus rotundus* and was first published in the book "The Famous Doctor's Record". It is pungent, slightly bitter, slightly sweet and flat in nature. It is used clinically for relieving liver stagnation, dysmenorrhea and abdominal pain, menstrual disorders, breast pain and abdominal pain due to qi stagnation. It has been described by traditional Chinese medicine practitioners as "the chief of qi diseases and the master of female medicine" and is a commonly used herb in Chinese medicine for clinical management of qi. At present, most of the research on nutgrass galingale rhizome is focused on the volatile oil part. The volatile oil of nutgrass galingale rhizome includes a variety of monoterpenes, sesquiterpenes and oxides, etc. Modern pharmacological studies have shown that the volatile oil of nutgrass galingale rhizome has anti-inflammatory and antipyretic effects. It has been proved that aromatic alkene and α-cyperone are the main components of the volatile oil of nutgrass galingale rhizome and both of them have strong biological activity[1].

The theory of Chinese medicine places great emphasis on the overall effect of Chinese medicine and the synergistic effect of various chemical components on the medicinal effect. Using only one or two "active ingredients" as quantitative indicators of Chinese medicine is far from reflecting the intrinsic quality of Chinese medicine as a whole. Therefore, the evaluation of aromatic herb by volatile oil alone and quality control by this means cannot reflect the quality of Chinese medicine in a comprehensive and scientific way. It is well known that the material basis for the efficacy of Chinese medicines is their intrinsic synergistic effect of various chemical components.

The herb contains a variety of components, including volatile oils, flavonoids, triterpenoids, sterols, alkaloids, etc. Little research has been reported on the other components of the herb other than volatile oils. In addition, the quality differences between the quality of nutgrass galingale rhizome from different origins and the quality differences between nutgrass galingale rhizome before and after vinegar roasting also need to be studied. Therefore, in order to control the quality of nutgrass galingale rhizome, it is necessary to study the components it contains, not only the volatile oil part but also the remaining parts. In this experiment, the chemical composition of the herbs of different origins as well as the quality of raw and vinegar herbs were investigated according to the available conditions to provide a basis for the quality control of the herbs.

MATERIALS AND METHODS

Determination of the volatile oil content of nutgrass galingale rhizome:

Apparatus: XS 225A analytical balance (Switzerland, d=0.1 mg); TC-15 thermostatic electric heating set (Haining Huaxing instrument factory); one set of volatile oil extractor and condensing unit. The water used was distilled water and other reagents were analytically pure. The herbs were purchased from Bozhou herbal market in Anhui Province and identified as dried rhizomes of *Cyperus rotundus* L. (Salviaceae) by Associate Professor Lei Long of the Department of Pharmacy, People's Hospital of Hubei Medical College. The samples are shown in Table 1. The vinegar of *Cyperus rotundus* was obtained by mixing the vinegar with additional vinegar produced in Shandong, smothering it, placing it in a pot, frying it to dry and then remove it to cool.

TABLE 1: SAMPLES OF NUTGRASS GALINGALE RHIZOME

Serial number	Sample name	Place of origin
1	*Cyperus rotundus*	Shandong
2	*Cyperus rotundus*	Anhui
3	*Cyperus rotundus*	Hainan
4	*Cyperus rotundus*	Sichuan
5	*Cyperus rotundus*	Guangdong
6	*Cyperus rotundus*	Henan

Take 15 g of each sample, weigh precisely and determine the moisture content according to the Chinese Pharmacopoeia, 2010 Edition, Appendix IXH using toluene method. Weigh precisely 60 g of the powdered aromatic herb and then place in a flask, add 600 ml of water and several glass beads, shake well and mix, then connect the volatile oil tester to the reflux condenser. Add water from the upper end of the condenser tube to fill the scaling part of the volatile oil tester and then overflow into the flask. Then set them, in the electric heating jacket to boil, keep slightly boiling for about 6 h, until the amount of oil in the tester no longer increases. Then stop heating, place for a moment, open the piston at the lower end of the tester and slowly release the water, set the upper end of the oil layer to reach 5 mm above the scale 0 line. After 1 h, open the piston and let the

oil layer fall until the upper end is exactly equal with the scale 0 line and then read the amount of volatile oil.

Determination of changes in the volatile oil content of nutgrass galingale rhizome before and after vinegar roasting:

The vinegar was obtained by mixing the additional vinegar from Shandong, smothering it, placing it in a pot, frying it, taking it out and letting it cool, and then use one sample of the vinegar as a sample to determine its volatile oil content before and after the vinegar sizzling by using the above method.

Determination of beta (β)-sitosterol content of nutgrass galingale rhizome:

Apparatus: Agilent 8453E Ultraviolet (UV)-Visible (Vis) spectrophotometer (USA); Diane P680 high performance liquid chromatograph; Hypersil GOLD™ aQ column (thermo, 250×4.6 mm); KQ-500 ultrasonic cleaner (Kunshan Ultrasonic Instruments Co., Ltd., power 500 W, frequency 40 kHz). β-sitosterol (the control) was purchased from China National Institute for the Control of Pharmaceutical and Biological Products (batch no. 110851-200504); chromatographic methanol (Dikma Co., Ltd.), the rest of the reagents were analytically pure. The herbs of *Cyperus rotundus* were purchased from Bozhou herb market in Anhui Province and identified by Associate Professor Lei Long of the Department of Pharmacy, People's Hospital of Hubei Medical College as the dried rhizome of *Cyperus rotundus* L. (Salviaceae). The source is shown in Table 1. Chromatographic column: Hypersil GOLD™ aQ column (thermo, 250×4.6 mm); mobile phase: Methanol; flow rate: 1.0 ml/min; column temperature: Room temperature; detection wavelength: 210 nm (fig. 1 and fig. 2).

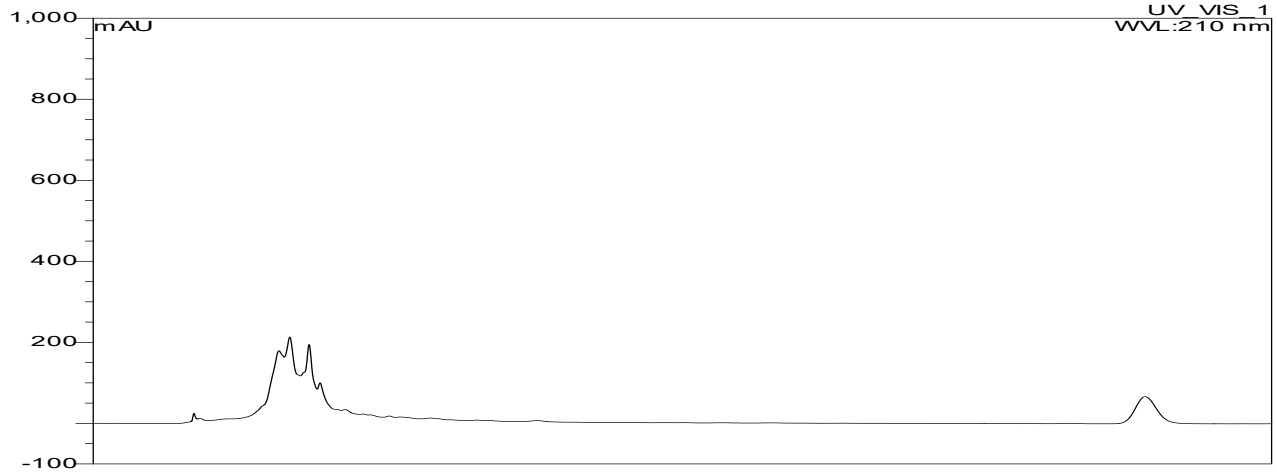

Fig. 1: UV-Vis spectra of β-sitosterol control

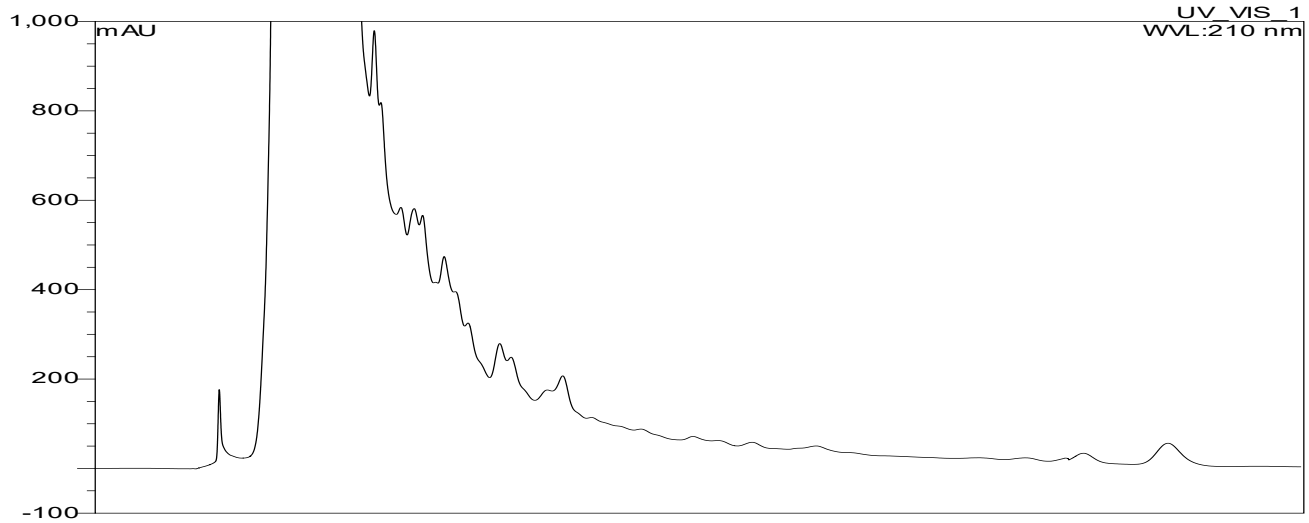

Fig. 2: UV-Vis spectra of aromatic herb samples

Take β-sitosterol control solution, use UV-Vis spectrophotometer to scan the full wavelength, where β-sitosterol has the maximum absorption wavelength at 210 nm. Weigh 5.0 mg of β-sitosterol in a 5 ml volumetric flask with methanol up to the mark and shake well. Take about 10 g of powdered aromatic herb, weigh precisely, put into a conical flask with stopper, add 100 ml of chloroform, extract by using ultrasonic-assisted extraction for 45 min, filter, then the filtrate is stored in another vessel, the filter residue is added with 80 ml of chloroform, extract with ultrasound for 30 min, filter again, then combine the two filtrates, concentrate and evaporate on a water bath, dissolve with methanol, fix the volume into a 2 ml volumetric flask, filter through a 0.45 μm microporous membrane and then take the renewed filtrate. Finally the solution was obtained. After examining the linearity, precision, repeatability and stability, the results obtained are good and then precisely aspirate 10 μl of each test solution. Inject the sample and calculate the β-sitosterol content by the method of external standard peak area.

Determination of changes in β-sitosterol content before and after vinegar roasting of nutgrass galingale rhizome:

The vinegar was obtained by mixing the additional vinegar produced in Shandong, smothering it, placing it in a pan, frying it and letting it cool. Three samples of aromatic herb from vinegar were taken and their β-sitosterol content was determined before and after vinegar roasting using the method described as above.

Determination of the total flavonoid content of nutgrass galingale rhizome:

Apparatus: UV-Vis spectrophotometer (Agilent 8453E, USA); BP211D electronic analytical balance (Sartorius, Germany); KQ-500 ultrasonic instrument (Kunming Ultrasonic Instruments Co., Ltd.); quartz cuvette; chromatographic column: 2.5 cm×14 cm; test drug is polyamide (30-60 mesh, Sijia Biochemical Plastic Factory, Luqiao, Taizhou, Zhejiang Province); rutin control (batch number: 10080-200306, purchased from China Institute for the Control of Pharmaceutical and Biological Products); water is redistilled water; methanol, ethanol, aluminium nitrate, sodium nitrite, sodium hydroxide and other reagents are all analytically pure. The herbs were purchased from Bozhou herb market in Anhui Province and identified by Associate Professor Lei Long of the Department of Pharmacy, People's Hospital of Hubei Medical College as the dried rhizome of *Cyperus rotundus* L. (Salviaceae). The samples are shown in Table 1.

Take 10 g of each sample, weigh precisely and determine the moisture content according to the Chinese Pharmacopoeia 2010 Edition, Appendix IXH using toluene method. Add 50 ml of petroleum ether (60°~90°) to a conical flask with a stopper, extract the sample with ultrasonic waves for 30 min, discard the petroleum ether and evaporate all the residual petroleum ether in the sample, add 70 % ethanol and extract the sample using ultrasonic extraction twice, add 45 ml each time, ultrasonic extraction for 45 min, let cool and filter the sample to obtain the filtrate. Weigh 3 g of polyamide with the sample solution and mix the sample. Dry the mixed polyamide overnight until it is free of alcoholic odour and load it onto the chromatographic column. The eluate was collected with 100 ml of distilled water, 50 ml of 20 % ethanol and 300 ml of 60 % ethanol, evaporated on a water bath and fixed to the mark to 25 ml with 60 % ethanol, shake well, then keep it for 6 min, add 4 % Sodium Hydroxide (NaOH) solution 4 ml, add 60 % ethanol to 10 ml, keep it for 15 min, then it is obtained.

Preparation of the control solution: Precisely weigh 20.08 mg of rutin (control) dried to constant weight, place in a 50 ml volumetric flask, add 60 % ethanol upto the mark and dissolve, shake well to obtain the solution. After examining the linearity, precision test, repeatability test and stability test, the results are found to be good. Take 3 g of *Curcuma aromatica* powder, weigh it precisely and prepare the test solution according to the preparation method and determine the absorbance value at 500 nm, deduct the water and calculate the content according to the dried product.

Determination of changes in the total flavonoid content of nutgrass galingale rhizome before and after vinegar roasting:

The vinegar was obtained by mixing the additional vinegar produced in Shandong, smothering it, placing it in a pot, frying it to dry and then removing it to cool. Three samples of nutgrass galingale rhizome in vinegar were taken and their β-sitosterol content was determined before and after vinegar roasting using the method as described above.

RESULTS AND DISCUSSION

Changes in the volatile oil content of nutgrass galingale rhizome of different origins and the volatile oil content of nutgrass galingale rhizome before and after vinegar roasting was shown here. The volatile oil content of the seven samples ranged from 1.13 % to 1.61 %, with the highest content in Anhui and Shandong nutgrass galingale rhizome, and the content of Shandong nutgrass galingale rhizome decreased from 1.59 % to 1.13 % after vinegar roasting as shown in Table 2.

β-sitosterol content of nutgrass galingale rhizome from different origins was shown here. The β-sitosterol content of the six samples ranged from 0.010893 % to 0.017923 %, with the highest content being in Anhui as shown in Table 3.

Changes in β-sitosterol content of nutgrass galingale rhizome before and after vinegar roasting was explained here. The β-sitosterol content was reduced in all three raw nutgrass galingale rhizome after vinegar roasting, as shown in Table 4.

Total flavonoid content of nutgrass galingale rhizome of different origins was explained here. The total flavonoid content of the six samples ranged from 0.95381 % to 0.63565 %, with the highest content being in Henan, as shown in Table 5.

Changes in total flavonoid content before and after vinegar roasting is as follow. The total flavonoid content of all three raw nutgrass galingale rhizome was increased after vinegar roasting. The results are shown in Table 6.

TABLE 2: VOLATILE OIL CONTENT OF NUTGRASS GALINGALE RHIZOME FROM DIFFERENT ORIGINS

Place of origin	Shandong	Sichuan	Guangdong	Anhui	Henan	Hainan	Vinegar making (Shandong)
Content (ml/g)	1.59 %	1.35 %	1.14 %	1.61 %	1.15 %	1.49 %	1.13 %

TABLE 3: β-SITOSTEROL CONTENT (%, g/g) IN NUTGRASS GALINGALE RHIZOME OF DIFFERENT ORIGINS

S. No of samples	Place of origin	Content	Relative Standard Deviation (RSD)
1	Shandong	0.013962 %	3.09 %
2	Anhui	0.017923 %	2.14 %
3	Hainan	0.012613 %	2.45 %
4	Sichuan	0.013713 %	1.12 %
5	Henan	0.011799 %	2.62 %
6	Guangdong	0.010893 %	1.97 %

TABLE 4: COMPARISON OF THE β-SITOSTEROL CONTENT OF RAW AND VINEGAR NUTGRASS GALINGALE RHIZOME

Group	Samples	Content	RSD
1	Raw aromatic herb	0.013466 %	2.21 %
	Balsam vinegar	0.011167 %	1.26 %
2	Raw aromatic herb	0.014157 %	2.52 %
	Balsam vinegar	0.011656 %	3.75 %
3	Raw aromatic herb	0.014262 %	1.25 %
	Balsam vinegar	0.010289 %	2.26 %

TABLE 5: TOTAL FLAVONOID CONTENT (%, g/g) IN AROMATIC HERB OF DIFFERENT ORIGINS

S. No of samples	Place of origin	Content	RSD
1	Shandong	0.63565 %	1.30 %
2	Anhui	0.76884 %	2.54 %
3	Hainan	0.75085 %	1.25 %
4	Sichuan	0.92793 %	3.11 %
5	Henan	0.95381 %	2.75 %
6	Guangdong	0.83611 %	1.94 %

TABLE 6: COMPARISON OF THE TOTAL FLAVONOID CONTENT OF RAW AND VINEGAR NUTGRASS GALINGALE RHIZOME

Group	Samples	Content	RSD
1	Raw aromatic herb	0.62831 %	1.24 %
	Balsam vinegar	0.64083 %	1.76 %
2	Raw aromatic herb	0.63402 %	2.56 %
	Balsam vinegar	0.65758 %	3.23 %
3	Raw aromatic herb	0.624461 %	2.78 %
	Balsam vinegar	0.70561 %	1.28 %

As a Chinese herb, nutgrass galingale rhizome has many effects such as sedation[2], protection of gastric mucosa[3], promotion of isolated lipolysis[4], hypolipidemia[5], hypoglycaemia[6], antibacterial[7] and anti-inflammatory, antioxidant and apoptosis. Experiments have shown that nutgrass galingale rhizome can prevent the invasion of free radicals into Deoxyribonucleic Acid (DNA), thus stopping the further spread of free radicals caused by ageing and cancer, and it is less expensive and less toxic when taken orally for a long period of time compared to chemical drugs, making it suitable for use in the development of anti-cancer drugs, so nutgrass galingale rhizome has great potential for development as an anti-cancer drug. The quality of the herb is closely related to its efficacy. It is a widely distributed species in the world, mostly found in the North and South regions of China, growing on mountain slopes and grasslands or by water, and is mainly produced in Shandong Province, such as Tai'an, Junan, Rizhao, etc. In addition, Songxian, Yichuan, Shanxi, Jiangsu, Zhejiang, Anhui, Hainan and other provinces also produce. Especially Hainan produce nutgrass galingale rhizome in large quantities and high quality production, accounts for about 2/3 of the total national production[8]. The aroma and quality of Shandong's aromatic herb is considered authentic and is known as the "East nutgrass galingale rhizome". Zhejiang production of aromatic herb quality is also good, customarily known as "South aromatic herb"[9]. There are currently no domestic species of nutgrass galingale rhizome and all commercial products are wild, with production relying mainly on natural resources. Concoction is a common method of processing Chinese herbs to improve their efficacy and to alter or enhance the site and tendency of their action. Vinegar roasting is the main method for the preparation of nutgrass galingale rhizome. After vinegar roasting, the leaching rate and volatile oil content of nutgrass galingale rhizome can be increased, thus improving the efficacy. In this study, the effects of different origins and vinegar roasting processes on the chemical composition of aconite were investigated in order to provide an effective basis for quality control of aconite.

The chemical composition of nutgrass galingale rhizome is complex, mainly containing volatile oil, triterpenoids, sterols, alkaloids, flavonoids, sugars, phenols, etc[10-12]. The volatile oil is one of the main active components of nutgrass galingale rhizome and is also the most studied component of nutgrass galingale rhizome, with a content of about 0.65 %-1.4 %[13], mainly including a variety of monoterpenes, sesquiterpenes and their oxides,

etc. Modern pharmacological studies have shown that the volatile oil of nutgrass galingale rhizome has anti-inflammatory and antipyretic effects. It has been experimentally proven that aromatic alkene and α-cyperone are the main components of nutgrass galingale rhizome volatile oil and both of them have strong biological activity. The present experiments showed that Shandong as a production area of nutgrass galingale rhizome, has a high content of volatile oil of nutgrass galingale rhizome. It is a manifestation of its neutral nature. The decrease in volatile oil content after vinegar sizzling may be due to the loss of volatile oil caused by heating during the vinegar sizzling process. At present, most of the clinical use of nutgrass galingale rhizome is of vinegar roasted type. This is consistent with the traditional Chinese medicine theory that the volatile oil content of Chinese herbs can be reduced after concoction, which reduces the irritation to the stomach and the volatile oil content of vinegar-roasted nutgrass galingale rhizome is lower than that of raw nutgrass galingale rhizome.

Sterols are extremely widespread in nature and although they are not abundant, they have high application value. Sterols are natural substances, non-toxic in themselves and have characteristics such as emulsification and stability, so they are widely used in medicine, food, cosmetics, animal growth agents, plant growth hormones and chemical industry, textile and other fields[14]. Since the discovery of β-sitosterol and soya sterol in cereals, people have conducted extensive and in-depth research on phytosterols, but few relevant reports on β-sitosterol and soya sterol in nutgrass galingale rhizome have been seen. The results of this experiment show that the content of β-sitosterol in nutgrass galingale rhizome is relatively low, basically not more than 0.015 %, but it has a large active effect. The β-sitosterol content of nutgrass galingale rhizome from Shandong and Anhui is higher than that of other nutgrass galingale rhizome. The β-sitosterol content of the nutgrass galingale rhizome was reduced after vinegar sizzling. Further research is needed to determine whether the changes in β-sitosterol content before and after vinegar sizzling are related to its medicinal effects.

Flavonoids have remarkable effects in the treatment of coronary heart disease, Alzheimer's disease, cerebral thrombosis, neurological disorders, antibacterial and anticancer, without side effects and as a result many kinds of medicines and health foods have been developed[15]. At present, there are relatively few studies on the flavonoids of nutgrass galingale rhizome at home and abroad. Only Huang *et al*.[16] used ultrasonic ethanol extraction method to extract flavonoids from aromatic herbs and verified the extracted flavonoids. The results showed that nutgrass galingale rhizome is relatively rich in flavonoids, therefore, nutgrass galingale rhizome has a wide range of development and utilization value and is worthy of comprehensive utilization and enhanced resource development. The results of this experiment showed that Shandong, as the authentic origin of nutgrass galingale rhizome, had the lowest total flavonoid content, suggesting that its total flavonoid may be related to its medicinal effect, but not much to its authenticity.

The increase in the total flavonoid content of nutgrass galingale rhizome after vinegar roasting may be due to the inactivation of some enzymes that degrade total flavonoids during the process of vinegar roasting, which is another aspect that justifies the clinical use of aromatic herbs mostly after vinegar roasting. The use of vinegar for the preparation of the nutgrass galingale rhizome is justified by the better medicinal properties of the herbs from Shandong and Anhui.

Author's contributions:

Mei Cao and Can Zhou contributed equally to this work. Mei Cao designed and performed the research and wrote the paper; Lijun Zhao designed the research and supervised the report; Huibin Yu designed the research and contributed to the analysis; Shanshan Wang, Linhai Wang, Zhengzheng Li and Ping Jin participated in these experiments and gave some good advice. All authors read and approved the final submitted manuscript.

Conflict of interests:

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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