

Antibacterial Activity of Anthraquinone from Aloe on Spiced Pig Head

Lingyi Xu^{1,2,3,4}, Xiao Li^{1,2,3,4}, Yuqian Cui^{1,2,3,4}, Meixia Pang^{1,2,3,4}, Fang Wang^{1,2,3,4} and Jinghua Qi^{1,2,3,4*}

¹College of Food Science and Engineering, Beijing Key Laboratory of Detection and Control of Spoilage Organisms and Pesticide Residues in Agricultural Products, Beijing 102206, China.

²Food Chemistry teaching team, Beijing University of Agriculture, Beijing 102206, China.

³Pig innovation team, Modern agricultural industry technology system Beijing innovation team, Beijing 100102, China. ⁴High Level Projects of Beijing Universities Cross Training, Beijing 102206, China

*Email: abc960718@sina.com

Abstract: [Objective] To optimize the extraction of anthraquinone from Aloe by ultrasonic extraction and its antibacterial activity. [Method] The influences of different extraction time and ethanol concentration, on anthraquinone content were evaluated by a single factor experiment. And anthraquinone content was determined by ultraviolet spectrophotometry. The bacteriostasis of anthraquinone on spiced pig head's common putrefying bacteria: Staphylococcus, Serratia, Bacillus, Proteus and the minimal inhibitory concentration (MIC) were studied by Oxford plate assay system. [Result] The best extraction time was 30 minutes and the best ethanol concentration was 80%. The antibacterial activity of the Aloe anthraquinone on Staphylococcus Aureus, Bacillus Proteus is obviously, the minimum inhibitory concentrations were 0.0625 g/mL, 0.05 g/mL, 0.125 g/mL respectively and no inhibitory effect on Serratia. [Conclusions] The anthraquinones from Aloe can inhibit a part of spoilage bacteria in spiced pig heads.

1. Introduction

Low-temperature meat products are popular with the public, because of its rich nutrition, strong flavor and rich sense of mastication. However, in order to maintain its flavor, the processing of sterilization conditions needs to be controlled at 68-70 degrees for 30 minutes. This leads to some spores and some heat-resistant bacteria are not all killed, and their higher water activity was also beneficial for putrefying bacteria to grow and reproduce, which causes that the low-temperature meat products are more perishable than other meat products and affects their shelf life [1-2].

To prolong the shelf life of low-temperature meat products, people generally choose to add some synthetic antibacterial agents, but with the improvement of living standards, people's health awareness gradually increased, more and more pollution-free, pollution-free natural products are demanded [3-9].

Aloe is a liliaceous plant, cold in property, bitter in flavor, rich in nutrients, available for daily consumption, and the main chemical are carbohydrate, anthraquinone compounds, enzyme (polypeptide), amino acids, vitamins and steroid compounds and other compounds [9-10]. The



anthraquinone from aloe has high medicinal value and can be bactericidal, antibacterial, antiinflammatory, detoxification, and promote wound healing, enhance immunity, beauty and so on.

In the food industry, the traditional method of anthraquinone from Aloe is used ethanol or alkaline aqueous solution to extract, but it has many disadvantages, such as many processes, large losses, long cycle, low yields of extraction. Ultrasonic extraction has many advantages, such as high extraction rate, easy operation, fast extraction speed and less loss of active components. It has been widely used in the extraction of natural products [11-15]. This experiment was used ethanol as extraction agent, and extracted the anthraquinones from Aloe by ultrasonic extraction method. The extraction of anthraquinones in Aloe was determined by using 1,8-dihydroxyanthraquinone as the reference substance. The extraction process was optimized. It can provide scientific basis for further research and production. The Oxford plate assay system was used to investigate the inhibition of anthraquinone compounds from Aloe to the main spoilage bacteria, *Staphylococcus*, *Serratia*, *Bacillus* and *Proteus* in spiced pig head, for the future, added the Aloe as a bacteriostatic agent into the low-temperature meat products to extend its shelf life.

2. Materials and Methods

2.1 Materials

Aloe block are purchased at Mengzheng Pharmacy. All organic reagents came from Beijing Land Bridge Company and all inorganic reagents came from Beijing Chemical Works. The indicator bacteria were isolated and purified from spiced pig head by Beijing University of Agriculture.

2.2 Alcohol Extract

Took out the dried Aloe block and grinded into powder by pulverizer, after 40 mesh sieve, it was placed in a sealed place at normal temperature.

2.2.1 Ethanol Concentration. The 2.5g samples were divided into five groups on average. Each group according to the solid-liquid ratio of 1:25, were added for 50%, 60%, 70%, 80%, 90% ethanol, soaking at room temperature, overnight. The samples were extracted by ultrasonic power 480W at 30 °C for 40 min. The extraction with ethanol was repeated thrice. The ethanol extracts were filtered and evaporated by using a rotary evaporator at 50 °C, kept at 4 °C.

2.2.2 Ultrasonic Time. The 2.5g samples were divided into five groups on average. Each group according to the solid-liquid ratio of 1:25, were added for 60% ethanol soaking at room temperature, overnight. Each group was extracted by ultrasonic power 480W at 30 °C for 20min, 30min, 40min, 50min, 60 min. The extraction with ethanol was repeated thrice. The ethanol extracts were filtered and evaporated by using a rotary evaporator at 50 °C, kept at 4 °C.

2.3 Ultraviolet Spectrophotometry

1,8-dihydroxyanthraquinone standard was accurately weighed 10mg and dissolved in 100mL methanol, which was the standard solution of 1,8-dihydroxyanthraquinone. Added 0.5 magnesium acetate-methanol solution as chromogenic agent, with 0.5 Magnesium Acetate-methanol solution as blank sample, the standard solution was spectrally scanned in 300-700nm [16], to determine the optimum absorption wavelength.

The standard solution was removed 0.5mL, 1.0mL, 1.5mL, 2.0mL, 2.5mL, 3.0mL, 3.5mL, 4.0mL and added 0.5 magnesium acetate-methanol solution as chromogenic agent, then constant-volume in 10mL volumetric flask. With 0.5 Magnesium Acetate-methanol solution as a blank solution, it was measured the absorbance. Absorbance (A) and concentration (C) was used as coordinates and fitted equation of linear regression.

2.4 Activation of Culture

Strains (*Staphylococcus*, *Serratia*ae, *Bacillus*, *Proteus*) were recovered to normal temperature and poured into 50mL sterilized nutrient broth, cultured 12 h at 30 ° C. And then the strain added to new nutrient broth medium with 2% inoculation amount, activated again.

2.5 Bacteriostat of Anthraquinone by Oxford Cup Method

The sterile nutrient agar was inoculated with microbial cells (1 mL of microbial cell suspension in 100 mL agar medium) at 46 ° C and poured into sterile petri dishes. Each dish was about 15-20mL.

The sterilized Oxford cup was placed on the carrier plate gently. 100 μ L of 1 g / mL of Aloe active ingredients and distilled water were added to the Oxford cups, respectively. Three sets of parallel observation were made. After incubating 12 h at 30°C, the antibacterial activity was evaluated by measuring the inhibition zones.

2.6 Determination of Minimum Inhibitory Concentration

1g / mL of Aloe active ingredients were diluted 2 times into different mass concentration gradient. 100mL Aloe extract of different concentrations were added in different Oxford cups on the same plate, and the purified distilled water was used as blank control. Three sets of parallel observation were made. After culturing 12 h at 30°C, the antibacterial activity was evaluated by measuring the inhibition zones. The lowest concentration of the extract that the inhibition zone was the same as the ck was regarded as MIC [17]. Data Processing software: SPSS 14.0.

3. Results and Discussion

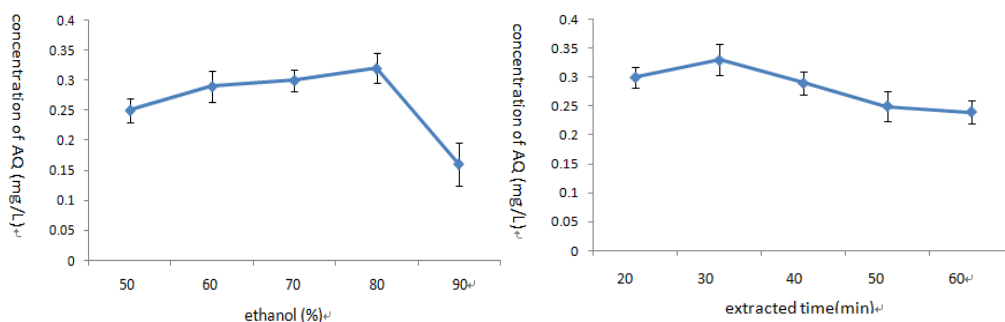


Figure 1. The effect of ethanol concentration extraction of Anthraquinone; **Figure 2.** The effect of extracted time on extraction of Anthraquinone

The as seen in figure.1, with the increase of ethanol concentration, the concentration of anthraquinone in the extract is also slowly increasing, and when the increase to 80%, the concentration of anthraquinone drops rapidly. When the concentration of ethanol was 80%, the concentration of the extract was the highest. The reason is that the form of anthraquinone in Aloe is conjugated anthraquinone glycoside and free anthraquinone, and the content of conjugated anthraquinone glycoside is higher. The conjugated anthraquinone glycosides are dissolved in water easily, while the free anthraquinone is soluble in organic solvent easily [18]. So when the ethanol concentration is less than 80%, with the increase of the concentration of ethanol, the free anthraquinone is soluble more. Total concentration of anthraquinone is gradually increased. Ut when the ethanol concentration is higher than 80%, anthraquinone glycosides which dissolved in water easily are dissolved decreased, making the concentration of the anthraquinone decreased.

The as seen in figure.2, before 30min, the concentration of anthraquinone increases with the increase of extracted time. After 30min, the concentration of anthraquinone decreases slowly with the increase of extracted time. The reason may be that some anthraquinones are unstable in heat. With the increase of extraction time, it will lead to decomposition [19].

Table 1. Inhibitory zone diameter of anthraquinonein Aloe

strain	Inhibitory zone diameter/mm
Proteus	12±0.2
Staphylococcus	15±0.5
Bacillus	12±0.2
Serratiaeae	0

Note: 0 means no inhibition zone

As can be seen from table 1, 1g/mL of anthraquinonein Aloe has obvious inhibitory effect on Proteus, Staphylococcus and Bacillus, but it has no bacteriostasis effect on Serratiaeae.

Table 2. MIC of anthraquinonein Aloe

anthraquinonein Aloe (g/mL)	1	0.05	0.25	0.125	0.0625	0.03125
Inhibitory zone diameter /mm	Proteus	12±0.2	10±0.1	0	0	0
	Bacillus	15±0.5	15±0.2	14±0.5	9±0.3	6±0.1
	Serratiaeae	12±0.2	12±0.4	12±0.2	6±0.1	0

Note: 0 means no inhibition zone

As can be seen from table 2, the anthraquinones in Aloe has well bacteriostasis effect on Staphylococcus which minimum inhibitory concentration is 0.0625g/mL and the maximum inhibition zone is 15mm; the effect of Bacillus is second which minimum inhibitory concentration is 0.125g/mL and the maximum inhibition zone is 13mm; the bacteriostasis effect on Proteus is ordinary which minimum inhibitory concentration is 0.5g/mL and the maximum inhibition zone is 12mm. The reason may be that Staphylococcus and Bacillus are gram positive bacteria, while Proteus and Serratiaeae belong to gram negative bacteria and the anthraquinonein Aloe is better for gram positive bacteria.

The components of anthraquinones in Aloe are complex. There are more than 20 kinds of substances [20] at least, such as aloin, Aloesin, Aloe-emodin and so on. The antibacterial mechanism of anthraquinonein Aloe might be two. One is to change the permeability of cell membrane leading to the cell damage: bacteria are through the selective permeability of cell to absorb nutrition from the outside world for its growth and reproduction. When the permeability of the cell membrane changing, the leakage of nucleic acids causes the cell deformation and nutrient flowing will result irreversible damage to bacteria themselves, and make bacterial die ultimately [21-22]. The other one is to inhibit the respiratory metabolism of bacteria: the growth and reproduction of bacteria cannot be separated from respiratory metabolism. Respiratory metabolism is the carbohydrates being oxidized and decomposed to release energy. Anthraquinone in Aloe can inhibit the Krebs cycle of oxidation of sugar and inhibit the synthesis of ATP and NADH in cell, which obstructs the oxidation of sugar. The respiratory metabolism of bacteria is affected, and then the bacteria can even lead to death [23-25].

4. Conclusion

In the single factor analysis of ultrasonic extraction, it can be concluded that 80% ethanol concentration is the best concentration of extraction and the extraction time of 30min is the best time. The main component of the extraction liquid is anthraquinone.

The anthraquinonein Aloe is better for gram positive bacteria in bacteriostasis effect. And with the decrease of the concentration of active ingredients, the inhibitory effect gradually decreased, which indicated that high concentration extracts have certain bacteriostatic effect.

5. References

- [1] Jos H.J and Huisin't Veld Microbial and biochemical spoilage of foods: an overview. International 1996 *Journal of Food Microbiology* **33** 1-18
- [2] Gram L and Dalgaard P Fish spoilage bacteria problems and solutions 2002 *Current Opinion in Biotechnology* **13** 262-266
- [3] Cao Yin Extraction process of anthraquinone from Aloe 2017 *TEXTILE AUXILIARIES* **34** 46-49.
- [4] W ANG Wei, WU Nan, FU Yu-Jie and ZU Yuan-Guang Antimicrobial Activities of Mentahaplocalyx Briq. Essentia Oil 2007 *BULLETIN OF BOTANICAL RESEARCH* **27** 626-629
- [5] Zhang guo quan Study on the extraction of the active substance from aloe barba densis and its antimicrobial effect 2011 *Shan Xi: Northwest A & F University* 1-2
- [6] ZHAI Mei-zhi, LI Xiao-wing, LIN QI-ying, XIE LIAN-hui Extract of inhibiting composition and inhibitory activity from walnut leaves 2003 *Journal of Northwest Forestry University* **15**(4) 89-91
- [7] LIU Zhi-xiong, WANG Li jun, YANG Ya zhen, BAI Xue and LI Ren jie Research on bacteriostasis effect of Aloe 2007 *JOURNAL OF SHANGHAI UNIVERSITY OF ENGINEERING SCIENCE* **21**(3) 219-221.
- [8] GANZLER K, SZONAL I and SALGO.A. Effective sample preparation method for extracting biologically active compounds from different matrices by a microwave technique 1990 *Journal of Chromatogr* **520** 257-262.
- [9] ZHANG Lei et al. Study on the Antibacterial Activity of Aloe Microwave Extracts 2012 *Journal of Anhui Ari. Sci* **40** (21) 11032-11034
- [10] ZHOU Mo-Lin, SHANGGUAN Xin-chen, ZHAN Ya-qiong and YANG Wu-ying Optimization of Ultrasonic-assisted Extraction for total Anthraquinones from Aloe Leaves by Response Surface Methodology F 2013 *Acta Agriculturae Universitatis Jiangxiensis* **35** (5) 1055-1062
- [11] Viljoen A M and Wyk B V. A Chemotaxonomic and morphological appraisal of Aloe series Purpurascetes, Aloe section Angui Aloe and their hybrid, Aloe broomii 2001 *Biochemical Systematic and Ecology* **29**(6) 621-631
- [12] Lambert R.J.W., Skandamis P.N., Coote, P.J., et al A study of minimum inhibitory concentration and mode of action of oregano essential oil, thymol and carvacrol 2001 *Journal of Applied Microbiology* **91** 453-462.
- [13] Vilkhuk K, Mawson R, Simons L, et al Applications and opportunities for ultrasound assisted extraction in the food industry a review 2008 *Innovative Food Science and Emerging Technologies* **9** (2) 161-169.
- [14] Wang J, Sun B, Cao Y, et al Optimisation of ultrasound-assisted extraction of phenolic compounds from wheat bran 2008 *Food Chemistry* **106** (2) 804-810.
- [15] Valero M. and Salmeron M C Antibacterial activity of 11 essential oils against *Bacillus cereus* in tyndallized carrot broth 2003 *International Journal of Food Microbiology* **85** 73-81.
- [16] Cha T L, Qiu L, Chen C T, et al. Emodin down-regulates androgen receptor and inhibits prostate cancer cell growth 2005 *Cancer research* **65**(6) 2287-2295.
- [17] Steenkamp V, Stewart M J. Medicinal applications and toxicological activities of Aloe products 2007 *Pharmacological Biology* **45**(1) 411-420.
- [18] Zhao Xia. Study on Extraction of the Anthraquinone from Aloe by ultrasonic method 2013 *Fiber and its Wide Watch* **13** 20
- [19] M Vmatoru. An overview- of the ultrasonically assisted extraction of bioactive principles from herbs 2001 *Ultrasonics Sonochemistry* **8** 303-313
- [20] L Panwnyk, E Bearfof, J P Lornmer and T J Nason. The extraction of rutin from flower buds of *Sophora japonica* 2001 *Ultrasonics Sonochemistry* **8** 299-301
- [21] Kieth Poole. Multidrug resistance in gram-negative bacteria 2001 *Current Opinion in biology* 500-508

- [22] Vivek K. Bajpai, Ajay Sharma and Bokyoung Moon Chemical Composition analysis and antibacterial mode of action of *Taxus cuspidata* leaf essential oil against foodborne pathogens 2014 *Journal of food safety* **34** 9-20.
- [23] National Committee for Clinical Laboratory Standards, Performance standards for antimicrobial susceptibility testing; ninth informational supplement 2008 *NCCLS document M100-S9* 120-126.
- [24] Wei Jin, Ying-Feng Wang, Ri-Li Ge, et al. Simultaneous analysis of multiple bioactive constituents in *Rheum tanguticum* Maxim. ex Balf. By high-performance liquid chromatography coupled to tandem mass spectrometry 2007 *Rapid Communications in mass Spectrometry* **21** 2351-2360.
- [25] Ultee A.. Adaptation of the food-borne *Bacillus cereus* to carvacrol 2000 *Archives of Microbiology* **174(4)** 233-238.