In vitro Evaluation of Antibacterial Activity and Protective Effects on Cardiomyocytes with H/R Injury of Garlic/Onion Oil

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Research

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Abstract

Background: At present, the research concerning *in vitro* experiments of garlic oil and onion oil is limited. The objective of this study was to carry out the research on the effective components of garlic and onion, and to study the antibacterial activity of the two essential oils and their protective effects on cardiomyocytes with hypoxia/reoxygenation (H/R) injury.

Method: The protective effect of volatile oils at different concentrations (200, 100, 50, 25, 12.5, 6.3, 3.1mg/L) on the endothelial cell of microvascular in cardiac muscle against H/R injury was examined by MTT and flow cytometer method. The survival rate and apoptosis situation were record. Inhibition zone test was used to evaluate antibacterial activity of volatile oils. MIC and MBC were calculated as well.

Results: To achieve a higher survival rate, the optimum concentrations of garlic oil and onion oil were 12.5mg/L and 6.13mg/L and high concentrations of garlic and onion oil decreased the survival rate. Both the 12.5mg/L onion and garlic oil reduced the apoptosis situation of cardiomyocytes. Both garlic oil and onion oil had antibacterial effect on gram-positive bacteria and gram-negative bacteria, and had the best antibacterial effect on cocci of gram-positive bacteria.

Conclusion: Garlic and onion oil do have antibacterial activity and protective effects on cardiomyocytes with H/R injury in a concentration range.

Background

In recent years, the studies on function of volatile oil extracted from plants have become a hot spot. It may have positive effects on the treatment of infectious diseases, protection of cardiovascular system and anti-oxidation. At the same time, it can also play a significant role in improving food quality. In addition, compared with synthetic substances, natural plant extracts have many advantages such as green, safe and environmental protection[1]. Volatile oil, also known as essential oil, is a general term for oil like liquid that can be volatilized at normal atmospheric temperature, can be distilled with steam and is not immiscible with water. In terms of chemical structure, it is mainly divided into terpenes, alkanes, alkenes, alcohols, esters, carbonyl and carboxyl compounds[2]. At present, the extraction methods of volatile oil at home and abroad include steam distillation, organic solvent extraction, supercritical carbon dioxide extraction and microwave-assisted extraction. Among them, steam distillation is the most commonly used method[3].

Garlic (*Allium sativum* L.) is a widely consumed spice and contains diverse bioactive compounds, such as alliin, allicin, diallyl trisulfide, diallyl disulfide, diallyl sulfide, ajoene, and s-allyl-cysteine. Substantial studies have shown that garlic and its bioactive constituents exhibit antioxidant, antibacterial, antifungal, anti-inflammatory, immunomodulatory, hepatoprotective, cardiovascular protective, anticancer, digestive system protective, anti-obesity, anti-diabetic, neuroprotective, and renal protective properties[4, 5]. Onion (*Allium cepa* Linn.) is also one of the oldest cultivated plants and is rich in nutrition. In addition, the content of carbohydrates, protein, water-soluble vitamins and calcium, iron, selenium is higher, and there

are sulfur compounds, flavonoids, steroids, prostaglandins and other physiological active materials. Onion has significant functions such as antioxidant, anti-cancer, anti-bacteria, reducing blood pressure, blood sugar, and blood fat[6, 7]. Garlic and onion belong to Allium family. Although they have different tastes and shapes, their biochemical and phytochemical compositions are similar and their sulfur compounds with strong flavor have antioxidant and antibacterial effects[8]. Studies have shown that garlic oil has a strong inhibition effect on many microorganisms which contaminated food. It has a wide antibacterial spectrum, mainly reflected in its obvious bacteriostatic and bactericidal effects on gram-negative bacilli such as *Escherichia coli, Pseudomonas aeruginosa, typhoid bacillus, tuberculosis bacillus;* gram-positive cocci such as *Staphylococcus aureus, pneumococcus, Streptococcus*, and fungi such as *Candida albicans*, etc[9, 10]. Onion oil has also been proved to have obvious inhibition effect on many gram-positive bacteria and gram-negative bacteria[11]. However, there are few comparative studies on the antibacterial effects of onion oil and garlic oil.

Nowadays, ischemic diseases are increasingly harmful to human health. Therefore, shortening the time of ischemia and restoring blood flow as soon as possible are the most effective measures to prevent and treat ischemic injury. However, in some special cases, when blood flow is restored, the damage to tissues and organs is always more serious, which is called ischemia/reperfusion injury[12]. This condition often occurs in the heart, brain, kidney and other important organs[13]. Therefore, the establishment of cell hypoxia reoxygenation injury model is of great significance in many aspects, such as ischemia/reperfusion, physiology, pathology, biochemistry, pharmacology and even clinic. Microvascular endothelial cell (MVEC) is a barrier between blood and tissue, and regulates and stabilizes the blood and tissue environment by secreting a variety of bioactive substances. At the same time, studies also show that MVEC is an important target cell of many toxins, viruses and other pathogenic factors[14]. At present, the antioxidant activity of onion and garlic is rarely studied by endothelial cells. Based on the special and important physiological function of endothelial cells, the hypoxia/reoxygenation (H/R) injury model of neonatal rat cardiac microvascular endothelial cells (CMEC) cultured *in vitro* was used to simulate the ischemia-reperfusion injury in vivo, and the protective effect of onion oil and garlic oil on the oxidative damage of CMEC was observed.

Methods

Materials

Staphylococcus aureus, *streptococcus hemolyticus*, *escherichia coli, pseudomonas aeruginosa, shigella flexneri, S. paratyphi B salmonella* and bacterial culture medium were provided by Center for Disease Control and Prevention of Jiangsu Province and Microbiology Department of Southeast University. Methyl thiazolyl tetrazolium, dimethyl sulfoxide, and sodium dithionite were purchased from Sinopharm Chemical Reagent Co., Ltd (Shanghai, China). Superior grade fetal bovine serum, endothelial cell growth additive, and heparin sodium were from Changzhou Xinhua Active Material Institute. Green streptomycin, penicillin and glutamine were received by Lianke Biotechnology Co., Ltd (Hangzhou, China). DMEM ordinary medium: 20% fetal bovine serum, 105 U/L penicillin, 100 mg/L streptomycin, 2 mmol/L L-

glutamine, DMEM medium; DMEM complete medium: 20% fetal bovine serum, 105 U/L penicillin, 100 mg/L streptomycin, 2 mmol/L L-glutamine, 50 mg/L heparin, 7.5 μg/L endothelial cell growth additive, DMEM medium. Onion oil and garlic oil were prepared according to the method[15].

Instruments

MCO-15AC CO_2 incubator was purchased from Sanyo Trading Co., Ltd (Japan), H_2S-H thermostatic water bath oscillator was from Harbin Donglian Electronic Technology Development Co., Ltd (Harbin, China), high-pressure steam sterilization pot and infrared sterilizer were purchased from Beijing Qincheng Scientific Instrument Co., Ltd, vortex mixer was from Gengchen Scientific Instrument Co., Ltd (Nanjing, China) and temperature and humidity regulating incubator was from Jinghong Experimental Equipment Co., Ltd (Shanghai, China).

Preparation of bacterial suspension

The freeze-dried bacteria tube was taken and open it under sterile conditions, appropriate amount of nutrient broth with capillary pipette was added, gently blew and sucked for several times to melt and dispersed the bacteria. The test tube containing 5.0~10.0 ml nutrient broth medium was taken, a little bacterial suspension was dropped and incubated at 37℃ for 18-24h.

The first generation of bacteria suspension was taken from the inoculation ring and inoculated on the nutrient agar medium plate, and cultured at 37℃ for 18-24h. The typical colonies of the second generation culture were selected and inoculated on the slant of nutrient agar and cultured at 37℃ for 18-24h, which was the third generation culture. The fresh slant culture (18-24h) of nutrient agar culture medium of the third to fourteenth generations of the strain and 3.0-5.0ml diluent with a 5.0ml pipette were taken, added it into the inclined tube, repeatedly blew and sucked, and the fungus coating was washed off. Then, the lotion was transferred to another sterile test tube with a 5.0ml pipette, mixed (oscillate) with an electric mixer for 20s, or vibrated 80 times on the palm of the hand to make the bacteria suspension evenly. The initial bacterial suspension was determined by turbidimetric method, and then diluted to 5×10^5 -5×10^6 cfu /ml.

Inhibition zone test

The qualitative filter paper was made into a round piece with a diameter of 6 mm by a punch. After high pressure sterilization, the qualitative filter paper was dried at 120℃ for standby. The treated filter paper was put into a clean plate, added onion/garlic oil diluent I 5μL, and should be used after natural drying at room temperature. At the same time, round filter paper containing n-hexane was made as control. The sterile cotton swab was dipped into the test bacterial suspension with the concentration of 5×10^5 -5×10^6 cfu /ml, and evenly applied on the surface of the culture medium plate for 3 times. The plate was rotated

60° for each application. Finally, the cotton swab was applied around the edge of the plate. The plate was covered and dried at room temperature for 5 min.

For each test, one bacterial plate was pasted, one test sample was pasted on each plate, and another negative control sample was made; or five test samples and one negative control sample were pasted on each plate, totally 6 pieces. The sample piece was pasted on the surface of the plate with sterile tweezers. The distance between the centre of each piece was more than 25mm, while the distance from the periphery of the plate was more than 15mm. After sticking, sterile tweezers were used to gently press the sample piece to make it close to the surface of the plate, then the plate was covered, and sample was incubated in 37℃ incubator for 24h to observe the bacteriostasis. When measuring the inhibition zone, the uniform and completely aseptic growth inhibition zone were chosen for measurement, calculated the diameter of the inhibition zone (including the patch) with digital vernier caliper, and recorded the measurement data. If the diameter of inhibition zone is more than 6 mm, it is judged that it has bacteriostatic effect. Otherwise it has no bacteriostatic effect.

Determination of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

One ml of onion/garlic oil diluent II was aspirated with sterile pipette gun and injected into a 90mm diameter aseptic culture dish, and then 15ml of dissolved medium (< 45℃) was added. After full mixing, the plate containing sample solution was prepared and numbered. Sterile water and 50% ethanol were used as control. 0.1ml of bacterial suspension or spore suspension on the surface of the plate was taken, evenly coated, incubated at 37℃ for 24h, and growth of the test bacteria was observed. The minimum inhibitory concentration of the tested sample was MIC. The plates of the above-mentioned non-growing bacteria were cultured for 24h. The minimum concentration of completely aseptic growth of onion/garlic oil was taken as MBC[16].

Culture of primary myocardial microvascular endothelial cells of rats

According to the method of Nishida[17], the method of in-vitro culture of intravascular endothelial cells in myocardial microvessels of neonatal rats was established. Four unborn 7~10d Wistar rats were killed by cervical dislocation, soaked in 75% alcohol for 2 minutes, and fixed with a pin. The chest was open, the heart was exposed, cut off and put into PBS solution, washed 3 times, the blood stains were washed, valves, large blood vessels and connective tissue with ophthalmology were cut off. The left ventricle was open and the heart was soaked in 75% ethanol for 30 seconds to inactivate the epicardial and endocardial endocardial epithelial cells. After rinsing with PBS solution, the myocardial tissue was cut up and then rinsed with PBS solution for 2 or 3 times. The washed tissue was precipitated with 2ml 0.2% type Ⅱ collagenase, blown, and incubated and digested 30min at 37℃. The same volume of pancreatic

egg albumin enzyme with a final concentration of 0.02% was added, the tissue was gently blew for 10 times and digested 10min in a hot water bath at 37℃. 4ml containing serum culture medium was added to stop digestion, the cells were separated through 200 mesh sieve, the precipitation without sieve was discarded, filtrate was taken, separated from the heart, repeated twice. The obtained cells were re-suspended in 8ml DMEM ordinary medium, blown evenly, inoculated in 1% gelatin-treated culture flask, cultured at 37℃ for 4 hours, to remove unadhered cells, and then replaced with DMEM complete medium containing 50mg/L heparin and 7.5μg/L endothelial cell growth additive. The liquid was changed once every 2 or 3 days, and the monolayer of cells was grown until the monolayer was grown. After being identified as CMEC, the cells were subcultured and the third generation cells were used in the experiment.

Establishment of hypoxia/ reoxygenation injury model

The original culture medium was absorbed and added into the non-serum DMEM culture medium containing 0.5mol/L and sodium disulfite, and incubated in a CO_2 incubator with a volume fraction of 5% at 37℃ for 60min (anoxic treatment). The culture plate was taken out, non-serum DMEM complete medium without sodium disulfite was added and continued to be incubated for 60min in a CO_2 incubator with a volume fraction of 5% at 37℃(reoxygenation treatment). The third generation neonatal rat myocardial microvascular endothelial cells were collected respectively, and the H/R model of isolated cells was established by hypoxia 1h/ reoxygenation 1h according to the above method.

Experimental grouping[18]

Myocardial microvascular endothelial cells were randomly divided into 11 groups with 5 holes in each group: (1) blank control group: DMEM complete medium cultured in saturated humidity carbon dioxide incubator for 2h; (2) solvent control group: DMEM complete medium containing 1‰ DMSO, cultured in saturated humidity carbon dioxide incubator for 2h; (3) model control group A: hypoxia for 1h and reoxygenation for 1h. (4) Model control group B: hypoxia for 1h and reoxygenation for 1h, then cultured in DMEM complete medium containing 1‰ DMSO. (5) garlic oil group (group 7): garlic/onion oil was incubated with 200, 100, 50, 25, 12.5, 6.3 and 3.1mg/L at the same concentration (DMEM complete medium) for 24h, then hypoxia for 1h and reoxygenated lh. They were also randomly divided into 11 groups with 5 holes in each group, and the garlic oil group was replaced by onion oil group, and the other groups were the same.

Determination of cell survival rate and apoptosis rate of cardiomyocytes

The absorbance (A value) measured by MTT method is positively proportional to the number of living cells, so the cell survival rate can be expressed as A value, and the inhibition rate = experimental hole A

value/control hole A value × 100%. The detection of MTT is completed on the enzyme-linked immunosorbent assay (Elisa).

Garlic oil and onion oil of 12.5mg/L were selected to detect the apoptosis rate of cells. After 6h of cell culture, the cells with precooled PBS were washed and resuscitated with diluted binding buffer and the cell density was adjusted to 5×10^5~1×10^6 per milliliter. 100μL cell suspension was taken into 5mL flow tube, AnnexinV- FITC 5μL and propidium iodide (PI, 20μg/mL) 5mL were added to cell suspension, gently shaken and incubated at room temperature and darked for 15 minutes, diluted binding buffer 400L was added into sample, and detected it on computer within 1h.

Statistical analysis

Results were expressed in the form of mean ± SD, statistical analysis was conducted by SPSS 19.0 software. The comparison between the two groups was conducted by t-test, and the comparison between multiple groups was conducted by analysis of variance (ANOVA). The comparison of inter group rates was performed by X^2 test, with statistical significance set $P < 0.05$.

Results

Antibacterial activity of onion oil and garlic oil by inhibition zone test

As shown in Table 1 and Table 2, the overall antibacterial effect of garlic oil may be much better than that of onion oil. There was no formation of bacteriostatic ring in control group. Onion oil had the best antibacterial effect on *Staphylococcus aureus, Escherichia coli* and *Shigella flexneri* followed by *Hemolytic streptococcus B*. Garlic oil had the best antibacterial effect on *Staphylococcus aureus* and *Pseudomonas aeruginosa*. What is more, the inhibitory effect of both garlic and onion oil on *Salmonella paratyphi B* was the lowest.

Table 1
Diameter of inhibition zone in onion oil (mm) ($\dot{x}$, n = 3)

Bacterial strains	Volatile oil					
	100%	50%	25%	12.5%	6.25%	3.13%
Escherichia coli	31.2	16.8	14.8	12.0	9.5	6.0
Shigella flexneri	28.9	24.1	17.7	13.2	9.4	6.0
Staphylococcus aureus	27.3	23.9	19.7	13.7	12.6	8.1
Streptococcus haemolyticus B	17.1	12.6	11.8	9.4	6.0	6.0
Pseudomonas aeruginosa	11.8	10.8	9.8	8.7	7.6	6.0
Salmonella paratyphi B	13.0	10.1	7.7	6.0	6.0	6.0
Control	6.0	6.0	6.0	6.0	6.0	6.0

Table 2
Diameter of inhibition zone in garlic oil(mm) ($\dot{x}$, n = 3)

Bacterial strains	Volatile oil					
	100%	50%	25%	12.5%	6.25%	3.13%
Staphylococcus aureus	>90	>90	>90	11.7	8.2	7.1
Streptococcus haemolyticus B	21.2	16.6	9.7	6.0	6.0	6.0
Pseudomonas aeruginosa	>90	>90	>90	8.5	7.3	6.0
Escherichia coli	18.0	10.2	7.8	6.0	6.0	6.0
Shigella flexneri	11.4	10.3	6.0	6.0	6.0	6.0
Salmonella	6.0	6.0	6.0	6.0	6.0	6.0
Control	6.0	6.0	6.0	6.0	6.0	6.0

Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

According to Table 3 and Table 4, bacteria still grew in sterile water and 50% ethanol control group. Onion oil had the strongest bactericidal effect on *Staphylococcus aureus, Escherichia coli* and *Shigella flexneri*, followed by *Streptococcus haemolyticus B*, while garlic oil had the strongest bactericidal effect on

Staphylococcus aureus, followed by *Pseudomonas aeruginosa* and *Streptococcus hemolyticus B*, but both of them not against *Salmonella paratyphi B*.

Table 3
MIC and MBC of onion oil ($\dot{x}$, n = 3)

Strains	MIC(%)	MBC(%)
Staphylococcus aureus	5.00	10.00
Escherichia coli	5.00	10.00
Shigella flexneri	5.00	10.00
Streptococcus haemolyticus B	10.00	> 10.00
Pseudomonas aeruginosa	—	—
Salmonella paratyphi B	—	—
Sterile water	—	—
50% ethanol	—	—
Note: "—" means no effect		

Table 4
MIC and MBC of garlic oil ($\dot{x}$, n = 3)

Strains	MIC(%)	MBC(%)
Staphylococcus aureus	1.25	2.50
Streptococcus haemolyticus B	5.00	10.00
Shigella flexneri	10.00	> 10.00
Pseudomonas aeruginosa	2.50	5.00
Escherichia coli	10.00	> 10.00
Salmonella paratyphi B	—	—
Sterile water	—	—
50% ethanol	—	—
Note: "—" means no effect		

Effects of garlic oil and onion oil on the survival rate of MVEC

After H/R injury of MVEC, absorbance and survival rate of the cells in model group were significantly decreased ($P < 0.01$). The cell survival rates of garlic oil and onion oil groups at 200 mg/L and 100 mg/L were significantly lower than those in the model groups ($P < 0.05$), and the cell survival rates of 25, 12.5 and 6.3 mg/L garlic and onion oil groups were significantly higher than those of the model groups ($P < 0.01$). The cell survival rate was the highest when garlic oil concentration was 12.5 mg/L and onion oil concentration was 6.3 mg/L. Garlic oil and onion oil can reduce H/R injury in a certain dose range, as shown in Table 5.

The cell survival rate of garlic oil group was significantly higher than that of onion oil group at 12.5 mg/L group, while the cell survival rate of onion oil group was significantly higher than that of garlic oil group at 25 mg/L and 6.3 mg/L group.

Table 5
The survival rates of cardiomyocytes with H/R injury affected by garlic and onion oil ($n = 5, \dot{x} \pm s$)

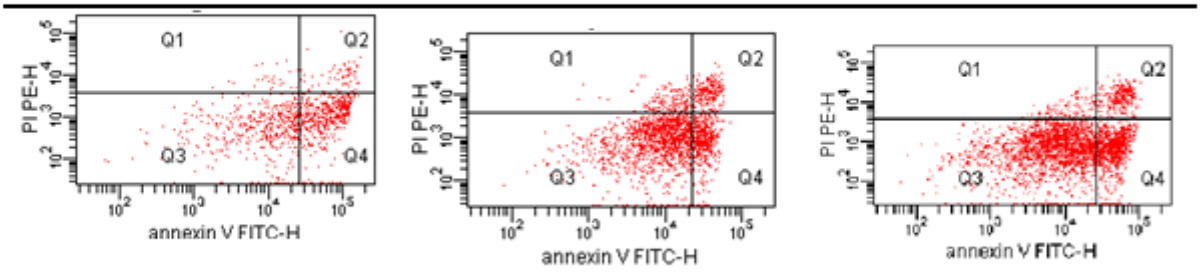
Group	Garlic oil	Onion oil	Comparison between groups
	survival rate(%)	survival rate(%)	
Normal control	99.05 ± 4.84	100.00 ± 1.35	P > 0.05
Solvent control	95.75 ± 5.18	91.68 ± 6.63	P > 0.05
Model control A	27.99 ± 3.22[a]	27.99 ± 3.22 [a]	P > 0.05
Model control B	27.82 ± 2.98[a]	27.82 ± 2.98 [a]	P > 0.05
200 mg/L group	20.80 ± 1.06[b]	11.70 ± 0.31 [b]	P < 0.05
100 mg/L group	21.84 ± 1.98[c]	12.48 ± 1.08 [b]	P < 0.05
50 mg/L group	23.40 ± 3.59	25.65 ± 3.67	P > 0.05
25 mg/L group	37.48 ± 3.49[b]	44.80 ± 7.23 [b]	P < 0.05
12.5 mg/L group	65.60 ± 4.77[b]	48.61 ± 5.79 [b]	P < 0.05
6.3 mg/L	42.46 ± 6.86[b]	55.37 ± 7.06 [b]	P < 0.05
3.1 mg/L group	27.64 ± 2.70	29.12 ± 1.51	P > 0.05
Note: Compared with normal control group, a means $P < 0.01$; compared with model control b group, b means $P < 0.01$,c means $P < 0.05$			

Effects of garlic and onion oil on apoptosis rate of MVEC

The apoptosis rate of garlic oil (12.5 mg/L) and onion oil (12.5 mg/L) were detected to explore the mechanism of their protective effect on MVEC injury induced by H/R. It can be seen from Table 6 that the apoptosis rates of garlic oil and onion oil group after H/R were 32.4% and 35.8%, respectively, which were significantly lower than those of the control groups. What is more, there was no significant difference in apoptosis rate between garlic oil and onion oil groups.

Table 6. The apoptosis situation of cardiomyocytes affected by garlic and onion oil

	H/R model	12.5mg/L garlic oil (%)	12.5mg/L onion oil (%)
Apoptosis rate	54.0	32.4[a]	35.8[a]



Note: Compared with hypoxia-reoxygenation model, a means P<0.05

Discussion

In recent years, people have been interested in a variety of phytochemicals and volatile oils extracted from phytochemicals. They have been used as one of the medicines to treat infectious diseases, and have also played a significant role in changing the quality of food. Natural plant extracts have many characteristics, such as green, safety, environmental protection and so on, which have gradually become the main antibacterial and antiseptic substances of the new generation. Because bacteria, mold and other microbial pollution are the main factors leading to food spoilage, preservatives have important application value in the process of food production, processing and storage. However, due to side effects of synthetic food additives, people tend to choose natural food preservatives with higher safety. At present, the research and development of natural food preservatives has become a hot spot at home and abroad.

The antibacterial effect of onion and garlic has been confirmed by increaseing number of studies, which has become a hot topic in recent researches. In this experiment, the antibacterial and bactericidal activities of onion oil and garlic oil were studied in vitro by inhibition zone test, MIC and MBC test. The results of inhibition zone test showed that garlic oil had bacteriostatic effect on *Staphylococcus aureus*, *Hemolytic streptococcus B*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Shigella flexneri*. Among them, the inhibitory effect on *Staphylococcus aureus* in gram-positive bacteria and *Pseudomonas aeruginosa* in gram-negative bacteria were the most ideal and on *Salmonella paratyphi B* of gram-

negative bacteria was the worst, which was consistent with the research results of Wu et al[19]. On the other side, onion oil also has a certain inhibitory effect on *Salmonella paratyphi B* in addition to the five strains above and has the most ideal bacteriostatic effect on *Staphylococcus aureus* and *Escherichia coli*, which is similar to the results of Jiang et al[20]. MIC and MBC results showed that garlic oil had the strongest bactericidal effect on *Staphylococcus aureus* with MIC value of 1.25% and MBC value of 2.5%, followed by *Pseudomonas aeruginosa* with MIC value of 2.5% and MBC value of 5%. And also showed the germicidal efficacy of onion oil on *Staphylococcus aureus*, *Escherichia coli* and *Shigella flexneri* was similar, MIC value was 5% and MBC value was 10%. All the experimental results were consistent with those of inhibition zone test. In conclusion, both garlic oil and onion oil have antibacterial effect on gram-positive bacteria and gram-negative bacteria, and have the best antibacterial effect on cocci of gram-positive bacteria.

The H/R model of neonatal rat CMEC showed that both garlic oil and onion oil had protective effects on the cells. According to the cell survival rate and anti-apoptotic effect, the cell survival rate of garlic oil and onion volatile oil was the highest in 12.5 mg/L and 6.3 mg/L, respectively. Garlic oil and onion oil of 12.5 mg/L had anti-apoptotic effect. In terms of mechanism, some studies suggested that allicin can achieve antioxidant effect through eNOS/NOZ signaling pathway, protecting H9C2 cardiomyocyte apoptosis and improving its activity[21]. Some studies also found that garlic extract can protect against possible heart injury by controlling heart sodium potassium pump and calcium level and reducing oxidative stress through chronic kidney injury model[22]. At the same time, quercetin and quercetin glucosides in onion have strong anti-platelet aggregation and antioxidant activities[23], quercetin can also inhibit the peroxidation of muscle satellite cells[24]; The polysaccharide extracted from onion also has certain antioxidant activity[25]. The results of this study can provide a scientific basis for the research of related products in the future. However, due to its complex mechanism of action, it may be affected by many factors, so the protective mechanism of CMEC against H/R injury needs to be further deepened. In addition, the results of this study showed that the cell survival rate of high concentration group decreased significantly, and there was significant difference compared with the model group. Considering that the higher the concentration of volatile oil, the stronger the direct killing effect of volatile oil on cells, and it was killed before induced apoptosis, so it had the effect of inducing apoptosis at high concentration. Studies have shown that, except for the cell necrosis caused by severe hypoxia in acute phase, most of the cells around the necrotic focus and damaged by chronic ischemia/reperfusion died in the form of apoptosis. However, in the early stage of myocardial reperfusion, CMECs are the first to undergo apoptosis, followed by cardiomyocytes, which suggests that the release of certain mediators by CMEC may affect cardiomyocytes apoptosis. Therefore, reducing the apoptosis of CMEC after reperfusion will play a positive role in the treatment of myocardial reperfusion injury[26].

Conclusion

Onion and garlic have a long history in China, which are traditional edible plants in China. Moreover. They have a pungent smell, which will affect the taste. Some people even do not eat garlic and onion at all. Therefore, the purpose of this project is to carry out the research on the extraction of effective

components of garlic and onion, and to study the antibacterial activity of the two essential oils and their protective effects on cardiomyocytes with H/R injury. On the one hand, it is conducive to expand the product sales market, meet the taste and health of consumers, improve the added value of special cash crops, increase farmers' income, and promote the change of agricultural industrial structure. On the other hand, it also provides broad prospects for the development and utilization of China's rich garlic and onion resources.

Abbreviations

CMEC, Cardiac microvascular endothelial cells;

MVEC, Microvascular endothelial cell;

H/R, Hypoxia/Reoxygenation;

MIC, Minimum Inhibitory Concentration;

MBC, Minimum Bactericidal Concentration;

Declarations

1. Ethics approval

   This study was approved by the ethics committee on animal research at Southeast University (20110701008).

2. Consent for publication

   Not applicable

3. Availability of data and materials

   The datasets used and/or analyzed during the current study are available from the corresponding author upon reasonable request.

4. Competing interests

   The authors declare that they have no competing interests.

5. Funding

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6. Authors' contributions

Yifei Lu (YFL)，Lv Hui (HL) and Lihua Li (LHL) designed this experiment. All the authors performed this experiment, Chao Yang (CY) and Ligang Yang (LGY) processed the data. YFL wrote the first draft of original manuscript , HL and Da Pan (DP) helped with the tables and figures. Professor GJS and SKW has revised the manuscript critically. All authors have read and approved the final manuscript. YFL is guarantor and had full access to all the data and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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