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Research Article

Research on the Antimicrobial Activity of α-triple Thiophene in the Marigold

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Abstract: In this study we measured the marigold extract α-triple thiophene by filter paper to study the kind of antibacterial activity in common food spoilage bacteria, the experiments found that the α-triple thiophene-like liquid has a descending antibacterial order of six kinds of bacteria as: the *Colibacillus*>*Penicillium*>*Salmonella*> *Staphylococcus aureus*>*B. subtilis*> root fungus. It had some inhibitory effect on *E. coli*, *Salmonella* and strains of *Penicillium*. And it had the most obvious inhibitory effect on the two kinds of subjects *E. coli* and the *Penicillium* and the minimum inhibitory concentration value is 15%.

Keywords: α-triple thiophene, antimicrobial activity, marigold

INTRODUCTION

Marigold (*Tagetes patul*), which is also known as Calendula, cellular (Chen, 2004) is an annual herb. *Tagetes*, height 60~100 cm, stout stems erect, much branched and smooth surface, leaves pinnatisect opposite, serrated leaf margin shallow, capitulum the top branches, stems around 10 cm, yellow or orange, long flowering period up to 8 to September, spend big and beautiful (Lin, 2002). In China Pharmacopoeia has long been documented, its flowers and leaves can be used as medicine, qi and blood root feasible, with high use value, such as antibacterial, treatment of toothache and repel insects and other effects. Modern pharmacological studies have shown that marigolds have Pinggan heat, anti-inflammatory, expectorant, blood, blood and new students, anti-tumor, enhance immunity and other effects (O Connell, 2004).

Marigold is a famous photoactivated insecticidal plants, photographic materials are mainly α-triple thiophene and its derivatives, has the same effect and absorption spectra. Was originally isolated from a chemical marigold, it only exists in marigold roots and flowers, the content of about 1 to 2% (Wang *et al*., 2004). Sunlight or ultraviolet light (300~400 nm), the half-life of α- thiophene as triple 4 h, environmentally safe, is expected to become a new antimicrobial agent. α-triple thiophene photoactivation has significant insecticidal activity (Baslas and Singh, 1981), plant pathogens, plant nematode has photoactivated insecticidal synergism (Le and Sheng, 1992). Current research on α-triple thiophene focused on the study of photochemical activity, without a definite extraction scheme and marigold extract lutein is mainly used, the residue after extraction majority are discarded as waste, causing great waste of resources, in order to better develop and utilize resources marigold, is exploring the residue α-triple thiophene extraction technology, comprehensive utilization of resources marigold will play an important research value (Fig. 1).

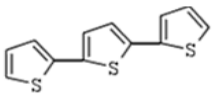

Fig. 1: Structure of α-terthienyl

Currently, people antibacterial activity of marigold α-triple thiophene limited to plant pathogens, such as pepper *Fusarium oxysporum* and antibacterial activity against common food spoilage bacteria rarely reported (Wang *et al*., 2011). This study is the first to marigold extract residue α-triple thiophene used to inhibit spoilage bacteria commonly cause food spoilage through trial found α-triple thiophene *E. coli*, *Salmonella* and *Penicillium* have strong antibacterial activity, would later cause food spoilage bacteria of other corrupt conduct research on the development of new food preservation, preservatives and other areas has a positive role in promoting.

TEST MATERIALS AND EQUIPMENT

Test material: α-triple thiophene crude extract: Ultrasonic assisted Soxhlet extraction preparation obtained (purity 49.52%); methanol, ethyl acetate, acetone, chloroform, sodium chloride: AR, Beijing Peng Cai Fine Chemical Co., Ltd.; *Penicillium*, *Escherichia coli*, *Staphylococcus aureus*: Institute of Food Microbiology Laboratory provided; *Salmonella*, *Rhizopus*, *Bacillus subtilis*: Institute of Food Microbiology Laboratory provided; peptone, sucrose, agar: biochemical reagents, Beijing double spin

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microbial culture products factory; glycerol: Food Grade, Zhengzhou Hongxiang Chemical Co., Ltd.

Test equipment: FA-1004 electronic balance: Shanghai Heng-Scientific Instrument Co.; HWS24 temperature water bath: Shanghai Scientific Instrument Co., a constant; BPX-82-type electric incubator: Shenyang Xin Jie Instrument Bose Limited; SHZ-D (III) circulating water pumps: Gongyi City English valley to China instrument; RE-52A rotary evaporator: Haiya Wing instrument; K-2500 CNC ultrasonic reactor: Kunshan Ultrasonic instrument Co., Ltd.; DZF-6021 vacuum oven: Suzhou Koto precision Instrument Co.; YX280D portable Pressure Steam autoclave: Hefei Huatai medical equipment Co., Ltd.; clean bench: Hal Beijing East instrument Co., Ltd.; vernier caliper: measuring instrument Co., Ltd. Kunshan traceable.

TEST METHODOLOGY

Preparation of the medium:

Potato medium: Potato weighing 200 g, Peel and wash broken into small pieces, add distilled water 1000 mL, boiled 30 min (Note fire control may be appropriate to pay), with gauze filter, make up the loss. Ten gram of sucrose was added to the filtrate, 20 g agar, heated to melt the agar, packed in tubes or flasks, high-pressure steam sterilization.

Broth (LB): Accurately weighed peptone, yeast extract, sodium chloride was 10, 5 and 5 g, respectively into a large beaker was added 700 mL of distilled water in a beaker larger than, were stirred with a glass rod, asbestos-line heated to melt, with 1 mol/LNaOH solution was adjusted to neutral pH, water poured into a graduated cylinder to the desired volume and finally add the agar 18 g, heated to melt, make up water up to 1000 mL. The dispensed medium was formulated into an Erlenmeyer flask and after dispensing, stuffed with cotton plug, the discharge medium only external bacterial contamination and good ventilation. Outsourcing medium and kraft paper and then tied good, high-pressure steam sterilization.

Preparation of bacterial suspension: Bacterial suspension of bacteria: *E. coli* in the refrigerator thermostat from 4°C and salmonella bacteria out of two subjects, activation on a slant medium, placed in a 37.5°C incubator, incubation 20~24 h. Then the received liquid medium slant strain placed 37.5°C, within 150 r/min shaking culture 4 h, was diluted out with a liquid medium to a bacterial concentration 1×106 CFU/mL, spare.

Fungal bacterial suspension: Lieutenant Green from the refrigerator at 4°C remove mold thermostat, on top slant medium, placed in 28°C incubator, incubation 40~48 h. Then wash with saline under the slant of bacteria, adding glass beads and shaker 10 min, diluted bacterial concentration to 1×106 CFU/mL, alternate with saline.

α-triple thiophene extract pretreatment: The three even-thiophene α-extract concentrated under reduced pressure to less than 20 mL extracts were placed in a vacuum oven for drying alternate. Weigh 1 g is α-triple thiophene extract, dissolved in 1 mL of deionized water to the saturation concentration of formulated into 1.0 g/mL of α-triple thiophene extract stock solution, stored at 4°C, the standby.

Determination α-triple thiophene-like liquid antibacterial effect: After the medium pressure steam retort sterilization, cooled to about 50°C, injected into a sterile petri dish and after cooling after curing, placed in the incubator. After the incubation period, remove the test bacteria which component, if the presence of other bacteria, use. In the clean bench, the first with a sterile pipette bacterial suspension 200 μL, were added to the culture medium surface, spread evenly, until the medium solidified, sterile filter forceps Magi 3, said bacteria onto the plate using a sterile pipette α- triple thiophene sample solution 20 μL 1.0 g/mL and added to the dish. Bacterial selection 37.5°C under the conditions of incubation 20~24 h; while 28°C under the conditions of mold selection, incubation 40~48 h. Vernier caliper with a diameter of inhibition zone sizes and recorded, the test three times in parallel. Its inhibition rate is calculated as follows:

$$\text{Inhibition rate}(\%)=\frac{\text{Sample handlinginhibition zone diameter-Blank sampleinhibition zone diameter}}{\text{Blank sampleinhibition zone diameter}}\times 100\%$$

Determination of the minimum inhibitory concentration: With 40% glycerol solution was diluted at a concentration of 1.0 g/mL of thiophene α-triple sample solution to a concentration of 5, 10, 15, 20, 25 and 30%, respectively of the mixed solution, with a sterile transfer pipette draw the prepared broth were 0.5 mL, corresponding to various concentrations of added diluent, with a 40% glycerol solution as blank control, fully mixed, placed in incubator and cultured 48 h, each concentration the test was done three times in parallel, remove the observations obtained minimum inhibitory concentration.

The relationship of thiophene inhibition rate and reaction time, concentration of α-triple mixture: Select α-triple thiophene bacteria mixture was tested for its inhibitory effect as bacteria, will α-triple mixture of thiophene select different processing times at different concentration conditions, even after the measurement process α-three thiophene mixed liquid antibacterial effect, using filter paper method, set the control group, will join α-triple thiophene dish into a thermostat incubator, bacterial selection under 37.5°C conditions, incubation 20~24 h; while fungal select 28°C under the

conditions of incubation 40~48 h. Every 2 h inhibition zone diameter measurements with calipers and record data.

ANALYSIS OF TEST RESULTS

Antibacterial measurement results: Determination α-triple thiophene-like liquid on different strains of inhibition zone diameter size using the paper method, the results shown in Table 1.

As apparent from Table 1, α-triple sample liquid thiophene is particularly strong inhibition of *E. coli*, followed by *Penicillium*, *Rhizopus* and finally *Bacillus subtilis*. α-triple thiophene-like liquid on *Staphylococcus aureus* and *Salmonella* inhibition similar, α-triple thiophene-like liquid antibacterial diameter is descending six kinds of bacteria: *E. coli*>*Penicillium*> *Salmonella*>*Gold aureus*>*B. subtilis*>root fungus.

The minimum inhibitory concentration measurement results: The best selection of antibacterial activity of *E. coli* and *Salmonella* and *Penicillium* strains tested, measured using filter paper extracts different dilutions of the minimum inhibitory concentration for different species and the results are shown in Table 2.

Table 2 shows, α-triple thiophene mixture of *E. coli*, *Salmonella* and three *Penicillium* strains tested were certain extent and the higher the concentration, the better antibacterial effect. Which the inhibition of *E. coli* and *Penicillium* most significant capabilities, including the minimum inhibitory concentration of 15%; while the ability of *Salmonella* more significant inhibitory effect, in which the minimum inhibitory concentration of 20%; experimental results show that, α-triple-thiophene mixture of *Escherichia coli* and *Penicillium* ability to inhibit higher antibacterial activity against *Salmonella*, *E. coli* and it will be as a representative of *Penicillium* species, its further study.

The relationship of thiophene inhibition rate and reaction time, concentration of α-triple mixture: The inhibitory effect of α-triple thiophene mixture on the relationship between *E. coli* and *Penicillium* time, concentration and inhibition rate are as shown in Table 3 and 4.

From Table 3 and 4, α-triple mixture of thiophene inhibitory effect with increasing concentrations of the test substance and the effect of time and the rise. In the same action time, α-triple mixture of thiophene concentration higher inhibitory effect is better; α-three with the same concentration of thiophene mixture, along with the prolonged duration of action of antimicrobial, antibacterial rate rise; under the conditions of the same concentration and the same action time, α-triple thiophene mixture of antibacterial activity against *E. coli* higher antibacterial activity of *Penicillium*.

Table 1: The antibacterial diameter of α-triple thiophene-like liquid/mm

Tested strains	1.0 g/mL (sample concentration)
Staphylococcus aureus	11.7
E.	14.2
Salmonella	12.1
Bacillus subtilis	10.3
Penicillium	13.5
Rhizopus	9.2

Table 2: The minimum inhibitory concentrations of three α-thiophene mixture even

	α-triple thiophene mixture concentration/%					
Tested strains	25%	20%	15%	10%	5%	25%
E.	-	-	-	+	+++	E.
Salmonella	-	-	++	+++	++++	*Salmonella*
Penicillium	-	-	-	++	+++	*Penicillium*

-: No colony growth; +: A small number of colony growth; +: There is not more than one-third of the plate area colony growth; +++: There are no more than half of the flat area of the colony growth; ++++: More than half of the flat area of the colony growth

Table 3: Effects of different time and concentration of α-triple thiophene on inhibition rate of *Escherichia coli*

	Inhibition rate/%				
Time/h	25%	20%	15%	10%	5%
20	73.6	65.4	30.6	10.1	0
22	75.3	68.1	29.9	10.4	0
24	76.5	68.5	30.2	10.8	0
26	76.8	68.7	31.3	11.3	0
28	78.4	68.4	32.1	13.2	0

Table 4: Effects of different time and concentration of α-triple thiophene on inhibition rate of penicillium

	Inhibition rate/%				
Time/h	25%	20%	15%	10%	5%
40	70.4	43.7	30.6	9.3	0
42	72.1	46.2	28.7	10.4	0
44	73.8	46.7	29.1	10.6	0
46	74.3	47.1	31.3	11.5	0
48	75.1	46.7	32.2	13.1	0

CONCLUSION

In this study we measured the marigold extract α-triple thiophene by filter paper to study the kind of antibacterial activity in common food spoilage bacteria, the experiments found that the α-triple thiophene-like liquid has a descending antibacterial order of six kinds of bacteria as: the *Colibacillus*>*Penicillium*> *Salmonella*>*Staphylococcus aureus*>*B. subtilis*>root fungus.

The α-triple thiophene had some inhibitory effect on *E. coli*, Salmonella and strains of *Penicillium*. And it had the most obvious inhibitory effect on the two kinds of subjects *E. coli* and the *Penicillium* and the minimum inhibitory concentration value is 15%.

The antibacterial effect of the α-triple thiophene mixture enhanced with the increasing concentrations of the test substance and the effect of time. When in a certain time, the antibacterial activity role of α-triple

thiophene mixture improved with the increasing concentrations of the test substance; When in a certain time, the concentration of the test substance, α-three antimicrobial activity even mixture of thiophene as the role of prolonged increase; when the test substance in the same concentration and time, α-triple thiophene mixture of antibacterial activity against *E. coli* is stronger than the antibacterial activity of *Penicillium*.

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