1 Introduction

Nowadays, olive oil (OO), pomegranate seed oil (PSO), and grape seed oil (GSO) are attracting growing interests due to their beneficial effects on human health. Numerous studies declared that OO, PSO, and GSO had anti-inflammatory, antioxidant, anticancer, cardiovascular protective activities, and so on. Self-nanoemulsifying system (SNES) is a transparent, thermodynamically stable system, which possesses self-nanoemulsifying capacity, able to convert into nanoemulsions below 100 nm upon exposure to water, gastric or intestinal fluid. Due to the high disperse property, both SNES and nanoemulsions are capable of enhancing the absorption of the oil as well as the nutrients with poor solubility in SNES. Apart from its advantages in increasing the intake of beneficial oils and nutrients, SNES may exert synergetic effect with the nutrients in the system. For example, if OO, PSO, or GSO serves as the oil phase of SNES, since the edible oil has diverse biological activities, it will act in parallel with the nutrients of the system to generate more potent therapeutic outcome, compared to the oil or nutrients alone.

For this consideration, the antioxidant activity of OO, PSO, and GSO from five sources was measured, respectively. The one with strongest activity in each type of oil was singled out as the oil phase to develop SNES. The changes in antioxidant and antibacterial activities when the oils were transformed into nanoemulsions, were studied in detail.

2 Materials and Methods
2.1 Materials and reagents

OO, an essential olive oil, was purchased from Xiuhua Plant Essential Oil Store (China). PSO was obtained from Xian Plant Biological Engineering Co., Ltd. (China). GSO was from Filippo Berio Company. The oils were tested to have the strongest antioxidant capacity among five sources and were used in the following experiments. Vitamin E
(VE) was purchased from Henan Aoyi Biological Technology Company (China).

Cremophor EL was obtained from Aladdin Industrial Corporation (China). The reagent α,α-diphenyl-β-picyrilhydrazyl (DPPH) was obtained from TCI Chemical Company (China). Other reagents, of analytical grade, were supplied by Chengdu Kelong Reagent Company (China).

2.2 Properties of the oils
2.2.1 Analysis of fatty acid composition
The methylation of OO, PSO and GSO, as well as the GC-MS analysis, were carried out according to the method reported by us before.

2.2.2 Determination of acid value, iodine value, saponification value and total polyphenols
The determination of acid value, iodine value, and saponification value of the oils was conducted following AOAC (2000). Iodine value was analyzed by Wijs iodine value. Total polyphenols was measured by Folin-Ciocalteau method. Briefly, 0.25 mL of oil was extracted with 0.5 mL of 80% ethanol (v/v) for three times. The ethanol extracts were combined together, from which 0.5 mL was drained out for the determination. The results were expressed as µg gallic acid equivalent (GAE)/mL oil.

2.3 Preparation of SNES
According to the preliminary experiments, Cremophor EL and polyethylene glycol 400 were selected as the emulsifier and co-emulsifier to prepare SNES, respectively. Oil, emulsifier, and co-emulsifier at the proportion of 1:4:4 was blended together to construct the SNES under constant stirring at 40°C. Before use, the SNES was diluted with 2 folds of water carefully, further diluted with 5 folds water to self-assemble into nanoemulsions. The sizes were measured by a photo correlation spectroscopy (Malvern Instruments, UK) to assert the self-nanoemulsifying property.

2.4 Changes in antioxidant activity
To assess the antioxidant activity of the oils against DPPH free radicals, DPPH solution was prepared with ethanol to make its absorbance at 517 nm in the range of 0.90 to 1.05. OO, PSO, and GSO of different volumes were drawn into tubes, diluted to 800 µL with ethanol, and mixed with 200 µL isopropanol and 2.0 mL DPPH, successively. The final concentrations of the oils ranged from 0.067% to 2.13% (v/v). After the solution stood in darkness for 30 min, the absorbance at 517 nm was determined (A), as reported by Wang et al. The blank control of 1 mL, in which ethanol replaced oil, was blended with 2.0 mL DPPH, and the absorption was assayed as mentioned above (A0). The scavenging rate was calculated according to the following equation:

\[
\text{Scavenging rate} = \frac{(A_0 - A)}{A_0} \times 100\%
\]  

To estimate the antioxidant capacity of SNES, DPPH was dissolved in ethanol and diluted with water to make its absorbance at 517 nm in the range of 0.90 to 1.05. SNES was also diluted with water to adjust its final concentrations equivalent to oil contents from 0.1% to 0.8% (v/v). SNES of 1 mL was mixed with 2 mL DPPH and the absorbance at 517 nm was measured (A). In addition, the background absorbance of the solution including 1 mL SNES and 2 mL solvent, was determined as well (A0). One mL of blank SNES controls exclusive of oil was mingled with 2 mL DPPH, and the determined absorbance was marked as A0. The scavenging rate was calculated as the following:

\[
\text{Scavenging rate} = \frac{(A_0 - (A - A_0))}{A_0} \times 100\%
\]  

2.5 Changes in antibacterial activity
Bacterial strains of Escherichia coli (E. coli, ATCC 10536), Bacillus subtilis (B. subtilis, ATCC 6633), and Yeast (ATCC 10231), representing Gram negative bacteria, Gram positive bacteria, and fungus, respectively, were obtained from School of Pharmacy and Bioengineering, Chengdu University. The disc diffusion method was applied to evaluate the antibacterial activities of the oils. Briefly, the oils were prepared into 80%, 40% and 20% solutions (v/v) with dimethyl sulfoxide (DMSO), respectively, from which 10 µL was drawn into a 7 mm filter paper disc. The density of the three bacterial suspensions was adjusted to 10^4 CFU mL^{-1} and 100 µL was spread on the agar plate, respectively. After the bacterial layer gelatinized, the filter paper discs were placed on it, cultured in a 37°C incubator for 24 h, and the inhibition zones were determined, respectively.

To assess the antibacterial strength of SNES, E. coli and B. subtilis were grown in 80% beef extract peptone medium. Yeast was cultured in 50% YPD medium including 0.2% agar. The density was adjusted to 10^4 CFU mL^{-1} SNES of OO, PSO, and GSO in different volumes were added to the tubes, mixed with 3 mL of bacterial suspension, and diluted with water to 5 mL, making the final concentrations of SNES in the range of 0.25% to 32% (v/v), respectively. Samples of E. coli and B. subtilis were incubated at 37°C for 12 h, while samples of Yeast were cultured in a water shaker for 8 h, with the temperature 37°C and shaking speed 150 rpm. When the time for the proliferation was over, the samples were blended with 1 mL sodium hypochlorite disinfectant, stood for 30 min, and the turbidity was measured, respectively. Besides, the experiment included the blank controls of SNES exclusive of oils and the negative control composed of water and bacterial suspension. The turbidity increment of the negative control was set as the reference of 100% viability. The viability of bacteria incubated with SNES and their blank controls was
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2.6 Statistical analysis
All experiments were repeated three times and the results were expressed as mean ± standard deviation (SD). The variations between data were considered significant when p < 0.05 by ANOVA analysis and Turkey test.

3 Results and discussion
3.1 Properties of the oils
3.3.1 Fatty acid composition
The fatty acid compositions of OO, PSO, and GSO were displayed in Table 1. It implied that OO, PSO, and GSO were primarily composed of unsaturated fatty acids. Total unsaturated fatty acid content in OO, PSO, and GSO was 76.71%, 87.39%, and 82.51%, respectively.

3.3.2 Acid value, iodine value, saponification value and total polyphenols
The results were shown in Table 2. It indicated that PSO stood distinctively from the other two oils. Its acid value was approximately 5 and 3 folds higher than that of OO and GSO. Moreover, polyphenol concentration in PSO was 2 and 10 times higher than that in OO and GSO, respectively.

3.2 Properties of SNES
All of the SNES exhibited excellent self-nanoemulsifying capacity, able to convert into nanoemulsions with the size beneath 25 nm upon exposure to 10 folds water. The diameter of nanoemulsions from OO SNES, PSO SNES, and GSO SNES was 23.16 ± 0.57 nm, 23.39 ± 0.17 nm, and 22.24 ± 0.36 nm, respectively. The polydispersity index (PDI) was 0.210 ± 0.023, 0.137 ± 0.008, and 0.154 ± 0.028, respectively, manifesting that the nanoemulsions were uniform.

3.3 Changes in antioxidant activity
Based on the scavenging rates of the oils and SNES against free radicals at different concentrations, the half clearance concentrations (CL50) were calculated (Table 3). The antioxidant activity was PSO > OO > GSO (p < 0.01), which was well consistent with their polyphenol contents. It was noteworthy that PSO showed even more powerful capacity over the control of VE in quenching DPPH (p < 0.05). SNES presented the same strength order.

SNES had no obvious impact on changing the antioxidant activity of OO and PSO, but notably boosted the capacity of GSO to scavenge DPPH free radicals (p < 0.05).

Table 1 The identification of fatty acids.

| No. | OO                     | PSO                     | GSO                     |
|-----|------------------------|-------------------------|-------------------------|
| 1   | Hexadecanoic acid (C16:0, 12.61%) | Hexadecanoic acid (C16:0, 3.54%) | Hexadecanoic acid (C16:0, 8.84%) |
| 2   | Linoleic acid (C18:2, 43.91%) | Linoleic acid (C18:2, 6.49%) | Linoleic acid (C18:2, 59.32%) |
| 3   | 9E-Oleic acid (C18:1, 30.38%) | 11-Oleic acid (C18:1, 6.38%) | 11-Oleic acid (C18:1, 22.05%) |
| 4   | 11-Oleic acid (C18:1, 1.83%) | Stearic acid (C18:0, 3.28%) | 9Z-Oleic acid (C18:1, 0.88%) |
| 5   | 9Z,12Z,15Z-Octadecatrienoic acid (C18:3, 0.33%) | Punic acid (C18:3, 73.93%) | Stearic acid (C18:0, 5.22%) |
| 6   | Stearic acid (C18:0, 5.49%) | 11-Eicosenoic acid (C20:1, 0.59%) | 9-Eicosenoate (C20:1, 0.26%) |
| 7   | 11-Eicosenoic acid (C20:1, 0.26%) | Eicosanoic acid (C20:0, 0.64%) | Eicosanoic acid (C20:0, 0.27%) |
| 8   | 18-Methylnonadecanoate (C20:0, 0.51%) |  |  |
| 9   | Docosanoic acid (C22:0, 0.68%) |  |  |

C18:2 indicates a C18 chain with two ethylenic bonds. The percentage contents were calculated from peak area ratios. OO, olive oil; PSO, pomegranate seed oil; GSO, grape seed oil.

Table 2 Physiochemical parameters of the oils.

| Oil   | Acid value (mg/g oil) | Iodine value (g/100 g oil) | Saponification value (mg/g oil) | Total polyphenols (μg GAE/mL oil) |
|-------|-----------------------|-----------------------------|----------------------------------|-----------------------------------|
| OO    | 0.30 ± 0.03a          | 113.86 ± 5.38              | 192.16 ± 5.51                    | 22.78 ± 0.20d                    |
| PSO   | 3.22 ± 0.01a          | 108.37 ± 9.33              | 191.10 ± 7.35                    | 70.93 ± 0.28e                    |
| GSO   | 0.58 ± 0.03b          | 103.07 ± 9.47              | 232.68 ± 4.08                    | 6.51 ± 0.09e                     |

The values were from triplicate assays and were expressed as mean ± SD. Different letters in the same column represent statistical significant differences (p < 0.05 in the column of acid value, p < 0.01 in the column of total polyphenols). GAE, gallic acid equivalent. Other abbreviations as Table 1.
In GSO are majorly composed of glycerides and phospho-oils, implying that GSO contained more esters. The esters of GSO have the highest saponification value among the three oils, which may favor the antioxidant components in GSO to contact with DPPH and yield more potent radical-quenching power.

### 3.4 Changes in antimicrobial activity

Since oil is liable to bring forth turbid solution, high background noise, and poor accuracy in absorbance measurement, the qualitative disc diffusion method was employed to assess the antimicrobial activity of the oils. On the contrary, as SNES is able to self-assemble into nano-emulsions upon exposure to large quantities of water and form clear solution, quantitative turbidity approach was applied in evaluating SNES capacity to inhibit bacterial growth.

The antibacterial activity of both the oils and the positive controls were displayed in Table 4. The solvent DMSO did not show any inhibition zone. Antimicrobial capacity of the oils increased in a concentration dependent manner. Compared to *B. subtilis* and Yeast, the three oils were more efficient in preventing the growth of Gram negative bacteria *E. coli*. The results were in line with other studies.

The antimicrobial activity of the SNES was shown in Table 5. SNES displayed the same trend as the oils that they were more powerful in suppressing *E. coli*. Meanwhile, SNES created totally different antibacterial profile from their oils. With respect to OO, PSO showed significantly higher power against *E. coli* at different concentrations (*p* < 0.05). However, when they were formulated into nanoemulsions, the activity of OO SNES in suppressing *E. coli* became slightly stronger than that of PSO SNES. Similarly, in spite of superior inhibitory effect of GSO over OO

### Table 3

| Sample | DPPH     |
|--------|----------|
| Oil    | OO 0.55%\(^b\) |
|        | PSO 0.20%\(^a\) |
|        | GSO 1.25%\(^x\) |
|        | VE 0.38%\(^b\) |
| SNES   | OO 0.49%\(^b\) |
|        | PSO 0.18%\(^a\) |
|        | GSO 0.8%\(^c, x\) |

The different letters imply the significant differences (*p* < 0.05). \(*\): *p* < 0.05, compared with the free oil of GSO. CL\(_{50}\), concentration of 50% clearance rate; SNES, self-nanoemulsifying system. Other abbreviations as Table 1.

### Table 4

| Sample          | Concentration | Diameter of inhibition zone (mm) |
|-----------------|---------------|----------------------------------|
|                 |               | *E. coli*                        | *B. subtilis*                  | Yeast                  |
| OO              | 20%           | 8.03 ± 0.15\(^b, x\)             | 8.03 ± 0.15\(^b, x\)           | 7.83 ± 0.12\(^b, x\)  |
|                 | 40%           | 8.63 ± 0.12\(^b, x\)             | 8.37 ± 0.12\(^b, x\)           | 8.10 ± 0.36\(^a, x\)  |
|                 | 80%           | 9.00 ± 0.15\(^b, x\)             | 8.37 ± 0.23\(^b, x\)           | 8.43 ± 0.15\(^b, x\)  |
| PSO             | 20%           | 8.80 ± 0.26\(^b, x\)             | 8.17 ± 0.06\(^b, x\)           | 8.07 ± 0.15\(^c, x\)  |
|                 | 40%           | 9.37 ± 0.21\(^b, x\)             | 8.50 ± 0.30\(^a, x\)           | 8.53 ± 0.15\(^b, x\)  |
|                 | 80%           | 10.20 ± 0.32\(^b, x\)            | 8.83 ± 0.02\(^a, x\)           | 9.17 ± 0.21\(^a, x\)  |
| GSO             | 20%           | 8.60 ± 0.26\(^a, x\)             | 8.13 ± 0.23\(^b, x\)           | 8.13 ± 0.15\(^c, x\)  |
|                 | 40%           | 9.20 ± 0.10\(^b, x\)             | 8.47 ± 0.21\(^b, x\)           | 8.67 ± 0.21\(^b, x\)  |
|                 | 80%           | 9.97 ± 0.21\(^a, x\)             | 8.83 ± 0.15\(^a, x\)           | 9.20 ± 0.10\(^a, x\)  |
| Cefotaxime sodium | 3 mg mL\(^-1\) | 15.20 ± 0.44                     | 16.00 ± 0.21                    | 13.70 ± 0.26          |
| Salicylic acid  | 20 mg mL\(^-1\) |                                    |                                 |                       |

The inhibition zone includes the filter paper disc with the diameter around 7 mm. The values were from triplicate assays and were expressed as mean ± SD. \(^+:\) comparison among different concentrations of the same oil. \(^a-b:\) comparison among the three oils at the same concentration. Different letters indicate the statistical significant differences (*p* < 0.05). Abbreviations as Table 1.
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4 Conclusion

SNES from OO and PSO had no evident impact on altering the antioxidant activity of the oils. However, SNES markedly enhanced the capacity of GSO to quench DPH free radicals (p < 0.05). PSO and GSO had similar antibacterial activities toward E. coli, B. subtilis and Yeast, which were generally more powerful than the activity of OO. Nevertheless, when they were prepared into SNES, the strength order against E. coli shifted to GSO SNES > OO SNES > PSO SNES and the capacity to restrain the growth of Yeast PSO SNES > OO SNES > GSO SNES. SNES reversed the original order of the oil antimicrobial activity. The study suggests that SNES exerts different impacts on the bioactivities of oils. The underlying mechanisms governing the oils and SNES to act on bacteria deserve future comprehensive study.

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Conflict of Interest

The authors declare no conflict of interest.

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Table 5 The IC₅₀ of the SNES against different strains of bacteria.

| Sample       | E. coli | B. subtilis | Yeast          |
|--------------|---------|-------------|----------------|
| OO SNES      | 15.23ᵇ  | 30.85ᵇ      | 24.38ᵇ         |
| PSO SNES     | 17.05ᵇ  | 24.13ᵃ       | 15.18ᵃ         |
| GSO SNES     | 11.56ᵃ  | 28.44ᵇ      | 25.86ᵇ         |
| Blank control| 27.02ᵃ  | Over 32ᵇ    | 31.15ᵇ         |
| Cefotaxime sodium (µg mL⁻¹) | 29.27 | 31.91 | 148.57 |
| Salicylic acid (µg mL⁻¹) | | | |

Different letters imply statistical significant differences (p < 0.05). IC₅₀, inhibitory concentration with bacterial viable rate of 30%. Other abbreviations as Table 3.
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