Study on the antibacterial activity of dandelion leaf tea

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Abstract. In this paper, Shandong dandelion tea was selected as the research object. Its water-soluble and fat-soluble components were extracted by alcohol precipitation method and Soxhlet extraction method, respectively. Antibacterial effects were tested on four different types of strains including \textit{Staphylococcus aureus} (S.A), \textit{Salmonella} (SE), \textit{Escherichia coli} (E. coli) and \textit{Candida albicans} (CA) in vitro by coating and drilling method. Results showed that dandelion tea powder had certain bacteriostatic effect on \textit{Staphylococcus aureus}, \textit{Escherichia coli}, \textit{Salmonella} and \textit{Candida albicans}. Water-soluble components mainly had bacteriostatic effect on \textit{Staphylococcus aureus}, \textit{Salmonella} and \textit{Escherichia coli}, and showed the strongest bacteriostatic effect on \textit{Staphylococcus aureus} but nearly no bacteriostatic effect on \textit{Candida albicans}. Fat-soluble components had obvious bacteriostatic effect on \textit{Candida albicans}, but not on \textit{Staphylococcus aureus} and \textit{Escherichia coli}.

1. Introduction

Dandelion, also known as popoding, is homologous plant food medicine, can be wholly edible and used as medicine. Furthermore, it has no drug resistance, and has certain effect on bacteriostasis, anti-inflammatory, heat clearing, detoxification, diuresis, disperse swelling and dissipate bindsand [1].

Results showed that water-soluble substances in dandelion, such as acid phenols, flavonoids and polysaccharides, had different degrees of antibacterial effect on \textit{Staphylococcus aureus}, \textit{Staphylococcus}, \textit{Salmonella}, \textit{Escherichia coli}, \textit{Salmonella}, \textit{Bacillus subtilis}, \textit{klebsiella pneumoniae}, \textit{Salmonella typhi} and etc [2-5]. Meanwhile, fat-soluble components of dandelion also have obvious antibacterial effect on \textit{Staphylococcus aureus}, \textit{Bacillus subtilis}, \textit{Escherichia coli} and \textit{Candida albicans}, Methicillin resistant \textit{Staphylococcus aureus} [6-7]. Although dandelion, like green tea [8], has a broad antibacterial effect [9], the main concern was focused on the dandelion plant itself. In production, 90\% of dandelion is used in the preparation of dandelion tea. It is not known whether the efficacy of dandelion tea has been lost through the process of tea green removing process, tea twistin and drying processes [10, 11], and researches on the antibacterial activity of dandelion leaf tea is still rarely reported domestically.

In this paper, superfine tea powder of dandelion leaves in Shandong Province was selected as the research object, and antibacterial effect of its fat-soluble and water-soluble components on four types of common pathogenic bacteria including \textit{Staphylococcus aureus}, \textit{Salmonella}, \textit{Escherichia coli} and \textit{Candida albicans} was studied [12-15]. The purpose is to provide reference for the efficient development of dandelion and the popularization and application of dandelion tea.
2. Materials and methods

2.1. Materials and reagents
Dandelion tea was purchased from Gengchen (Shandong) Agricultural Technology Co., Ltd. Dandelion leaf tea with 200-mesh was selected in the experiments. Other reagents and materials used were all listed as Table 1 and Table 2.

Table 1. Main reagents in the experiments

| Reagents                                                      | Grade |
|---------------------------------------------------------------|-------|
| Absolute Ethyl Alcohol; Petroleum Ether; Dimethyl Sulfoxide   | AR    |
| (DMSO); Sodium Chloride                                       |       |
| Aagar Powder; Nutrient Agar; Beef Extract; Peptone            | BR    |
| Broth (LB); Nutrient broth (NB)                               | BR    |
| Liquid Sabouraud Medium                                       |       |

Table 2. Experimental strains and their corresponding positive control in the antibacterial experiments

| Experimental strains | Positive control                                    |
|----------------------|-----------------------------------------------------|
| *Escherichia coli* (E. coli); *Staphylococcus aureus* (S. A) | Pudilan antiphlogistic oral liquid |
| *Salmonella* (SE)   | Houerhuan antiphlogistic grains                     |
| *Candida albicans* (CA) | Clotrimazole vaginal tablets                      |

2.2. Instruments and equipment
Names and Models of the instruments and equipment used in the experiment were illustrated in Table 3.

Table 3. Instruments and equipment

| Name of instruments and equipment        | Model of instruments and equipment |
|------------------------------------------|-----------------------------------|
| Vertical pressure steam sterilizer       | BXM-30R                           |
| Super-clean worktable                    | VD650                             |
| Optical microscopy                       | N-10E                             |
| Ultraviolet-visible spectrophotometer    | T9S                               |
| Constant temperature incubator           | SPDX-400                          |
| Constant temperature drying g box        | DHG-9005                          |
| Whirlwind mill                           | JXFM110                           |
| Ultrasonic cleaner                       | KQ-250B                           |
| Vacuum drying oven                       | DZF                               |

2.3. Extraction of fat-soluble substances from dandelion leaf tea
Soxhlet extraction method was used to extract the liposoluble components in the dandelion leaf tea. Firstly, the dandelion leaf tea was dried and crushed into powder in a whirlwind mill, and was sifted through 200-mesh sieves. Then 3 accurate weight of tea powders samples (~15 g each) in filter paper cartridge were putted in Soxhlet extractor and extracted with petroleum ether (boiling point: 30–60 °C) at 60 °C for about 10 h. After thorough extraction, petroleum ether was recovered, and the yellowish-green fat-soluble components of dandelion leaf tea could be obtained which would be diluted with DMSO [16].

2.4. Extraction of water-soluble substances from dandelion leaf tea
Dandelion tea powder about 150 g and 10-16 fold of water was soaked for 0.5 h, stired while heated up to boiling, filtered after 1.5 h and the filtrate was collected. Then the process was repeated using another 7-8 fold of water and the filtrate was combined and concentrated. After that, anhydrous ethanol was added into the concentrated solution to make the ethanol content reach 70% in the final solution, after 24 h, the first alcohol precipitation was conducted. The process was conducted once again to make the
ethanol content reach 80% in the final solution after the ethanol was removed with distilling. At last, the sample was diluted to 50 mL with water and was stored at 4–8 °C for use [17].

2.5. Activation of culture

*Staphylococcus aureus*, *Salmonella* and *Escherichia coli* were cultured in the sterilized nutrient broth in constant temperature incubator at 37 °C for 24 hours [18], then the bacterial concentration was initially adjusted to 10^8 CFU/mL by McFarland turbidimetric method [19], and the bacterial suspension concentration was compared by plate counting method. To facilitate experimental operation, the concentration of bacterial suspension was further adjusted according to Xiao *et al.* which used the absorbance at 600 nm as reference [20].

*Candida albicans* was inoculated in a sterile Sabouraud's liquid medium and cultured in a constant temperature shaking incubator at 37 °C with a speed of 200 r / min for 8–9 h. The concentration of *Candida albicans* was adjusted to 10^8 CFU/mL. The concentration of bacterial suspension was also adjusted taking the absorbance at 600 nm as reference.

2.6. Bacteriostatic test

Bacteriostatic test was conducted with coating and drilling method. Beef extract peptone or LB agar medium was sterilized at 121 °C for 20 minutes and the plates were prepared on the sterile operating platform. After the medium in the plate is solidified, 100 μL of bacterial solution was coated evenly on the plate with a coating rod. After the bacterial solution was completely absorbed, a punch of 6 mm was used to punch holes equidistantly on the plate, then different concentrations of extraction solution, blank control solution and positive control solution were injected into each well, cultured in 37 °C incubator for 18 ~ 36 h and the size of inhibition zone was observed and recorded. In this experiments, Pudilan antiphillogistic oral liquid (1g/mL), Houerhuan antiphillogistic grains (1g/mL), and Clotrimazole vaginal tablets (0.1g/mL) were used as positive control for *Escherichia coli* (*E. coli*) and *Staphylococcus aureus*, *Salmonella* and *Candida albicans* respectively (Table 2). Water and DMSO was used as blank control for antibacterial activity of water-soluble and fat-soluble components in dandelion leaf tea.

2.7. Determination of minimal inhibit concentration (MIC)

Double dilution method: Eight testing tubes of 8 mL was numbered and was sterilized in sterilizer. After sterilization, 2 mL of sterilized LB broth medium was added to each test tube on the sterile operating platform, and 2 mL of dandelion leaf tea extract with appropriate concentration was added to one of the above tubes. After mixing, 2 mL of the solution was taken out from the tube and was added to another test tube. Repeat the above operation until the solution was diluted in the seventh tube. The eighth tube was used as positive control. Then 0.2 mL of the corresponding bacterial solution was added to the eight test tubes in turn, and cultured in incubator at 37 °C for 24 h. If the LB broth was turbid, it indicated that the results were positive and the concentration of the extract has no antibacterial effect and conversely instead. The minimum concentration with antibacterial effect was recorded as MIC [21].

2.8. Determination of minimum bactericidal concentration (MBC)

Clear, transparent and sterile broth was selected from the above tube, one ring was taken from each ring from inoculation ring, and then it was inoculated onto the sterile LB solid medium, and was cultured continually in incubator at 37 °C for 48 h. The minimum concentration corresponding with aseptic growthhand on the plate was record as MBC [21].

3. Results

3.1. Antibacterial effects of water-soluble components of dandelion leaf tea

Four different types of common pathogenic bacteria were selected to investigate antibacterial effects of water-soluble components of dandelion leaf tea. Their inhibition effects for the four different strains were listed in Table 4 in terms of the size of bacterial inhibition zone [22]. We can see that water-soluble
components of dandelion leaf tea showed different antibacterial effects for four types of strains. For S.A, SE and E. coil, the results were positive, while for CA, the results were negative. This indicated that water-soluble components of dandelion leaf tea showed antibacterial activity for S.A, SE and E. coil, but it had no bacteriostatic effect for CA. It can be seen that at the concentration of 2.5 g/mL and 2.0 g/mL, the antibacterial effects of water-soluble components of dandelion leaf tea for S.A and E. coil was of the same degree of that of positive control, to which S.A and E. coil was highly sensitive and moderately sensitive, respectively. This was mainly because that dandelion leaf tea was rich in flavonoids, polysaccharides, polyphenols, organic acids, etc [10], which showed antibacterial effect on Staphylococcus aureus and E. coil [2-4]. For SE, the antibacterial effects of water-soluble components of dandelion leaf tea was higher than that of the positive control when its concentration was higher than 1.5 g/mL, this could be attributed to phytic acid, a water soluble substance which could inhibit the growth of Salmonella by destroying the cell membrane of Salmonella [9].

Table 4. Antibacterial effect of water-soluble components of tea leaves for four different types of strains

| Concentration (g/mL) | 3.00 | 2.50 | 2.00 | 1.50 | 1.00 | 0.50 | 0.00 | Positive control |
|----------------------|------|------|------|------|------|------|------|------------------|
| S. A                 |      |      |      |      |      |      |      |                  |
| Diameters of bacterial inhibition zone (mm) | 16.5 | 15.0 | 14.5 | 13.0 | 12.0 | 9.0  | 6.0  | 16.0             |
| Relative sensitivity | +++  | +++  | ++   | ++   | +    | -    | +++ |                  |
| SE                  |      |      |      |      |      |      |      |                  |
| Diameters of bacterial inhibition zone (mm) | 12.0 | 12.0 | 11.5 | 10.0 | 8.0  | 6.0  | 6.0  | 9.0              |
| Relative sensitivity | ++   | ++   | ++   | ++   | +    | -    | -    | +                |
| E. coil             |      |      |      |      |      |      |      |                  |
| Diameters of bacterial inhibition zone (mm) | 10.5 | 10.0 | 10.0 | 8.0  | 6.5  | 6.0  | 6.0  | 10.0             |
| Relative sensitivity | ++   | ++   | ++   | +    | -    | -    | -    | ++               |
| CA                  |      |      |      |      |      |      |      |                  |
| Diameters of bacterial inhibition zone (mm) | 6.0  | 6.0  | 6.0  | 6.0  | 6.0  | 6.0  | 6.0  | 21.5             |
| Relative sensitivity | -    | -    | -    | -    | -    | -    | -    | +++              |

Notes: The sensitivity of the strains can be categorized in to 5 degrees, that is: Extremely sensitive “++++” (D ≥ 20 mm); Highly sensitive “+++” (15 mm ≤D<20 mm); Moderately sensitive “++” (10 mm≤ D<15 mm); Lowly sensitive “+” (7.8mm≤D<10 mm); Ineffective”-” (D≤7.8mm) [22].

3.2. Antibacterial Effects of fat-soluble components of dandelion leaf tea

The antibacterial effects of fat-soluble components of dandelion leaf tea had been illustrated in Table 5. Results showed that fat-soluble components of dandelion leaf tea had well bacteriostatic effect on CA, but nearly had no bacteriostatic activity for S.A, SE and E. coil the results were inconsistent with that reported previously by Duan, who demonstrated that fat-soluble components of dandelion leaves showed obvious antibacterial effect on S.A [7]. It can be inferred that in the process of frying tea from dandelion leaves, the loss of fat-soluble antimicrobial components might lead to the weakening of their antibacterial effect. Therefore, the control of heating temperature and time is very important for the efficacy of dandelion tea.
Table 5. Antibacterial effect of fat-soluble components of tea leaves for four different types of strains

| Concentration (g/mL) | 3.00 | 2.50 | 2.00 | 1.50 | 1.00 | 0.50 | 0.00 | Positive control |
|----------------------|------|------|------|------|------|------|------|-----------------|
| S. A                 |      |      |      |      |      |      |      |                 |
| Diameters of bacterial inhibition zone (mm) | 6.0 | 6.0 | 6.0 | 6.0 | 6.0 | 6.0 | 6.0 | 16.1            |
| Relative sensitivity | -   | -   | -   | -   | -   | -   | -   | +++             |
| SE                   |      |      |      |      |      |      |      |                 |
| Diameters of bacterial inhibition zone (mm) | 7.0 | 6.20 | 6.0 | 6.0 | 6.0 | 6.0 | 6.0 | 10.0            |
| Relative sensitivity | -   | -   | -   | -   | -   | -   | -   | ++              |
| E. coli              |      |      |      |      |      |      |      |                 |
| Diameters of bacterial inhibition zone (mm) | 6.0 | 6.0 | 6.0 | 6.0 | 6.0 | 6.0 | 6.0 | 9.7             |
| Relative sensitivity | -   | -   | -   | -   | -   | -   | -   | +               |
| CA                   |      |      |      |      |      |      |      |                 |
| Diameters of bacterial inhibition zone (mm) | 14.0 | 13.0 | 13.0 | 12.5 | 11.0 | 10.0 | 6.0 | 22.0            |
| Relative sensitivity | ++  | ++  | ++  | ++  | ++  | ++  | -   | +++++           |

3.3. MIC and MBC of water-soluble components in leaf tea for different strains.
Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of water-soluble components in leaf tea for the four different types of strains had been listed in Table 6. The MIC followed the order: S.A < SE and E. coli < CA. The MBC followed the order: S.A < E. coli < SE < CA. So antibacterial and bactericidal effect of water-soluble components in leaf tea showed opposite trend with the best antibacterial and bactericidal effect for Staphylococcus aureus, and had the worst antibacterial and bactericidal effect for CA. The results were coincident with the diameters of bacterial inhibition zones obtained in Table 4.

Table 6. MIC and MBC of water-soluble components of tea leaves for four different types of strains

| Four different strains | S.A | SE | E. coil | CA |
|------------------------|-----|----|---------|----|
| MIC (g/mL)             | 0.03125 | 0.1875 | 0.1875 | >3.00 |
| MBC (g/mL)             | 0.25 | 0.75 | 0.375 | >3.00 |

3.4. MIC and MBC of fat-soluble components in leaf tea for different strains.
Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of fat-soluble components in leaf tea for the four different types of strains had been listed in Table 7. The MIC followed the order: CA < SE < S.A and E. coli. The MBC followed the same order of MIC. So antibacterial and bactericidal effect of fat-soluble components in leaf tea showed the best antibacterial and bactericidal effect for CA. The results were coincident with the diameters of bacterial inhibition zones obtained in Table 5.

Table 7. MIC and MBC of fat-soluble components of tea leaves for four different types of strains

| Four different strains | S.A | SE | E. coil | CA |
|------------------------|-----|----|---------|----|
| MIC (g/mL)             | >3.00 | 0.375 | >3.00 | 0.0625 |
| MBC (g/mL)             | >3.00 | 1.50 | >3.00 | 0.25 |

4. Conclusion
Dandelion tea which was harvested, cleaned and dried from dandelion leaves, showed antibacterial effects on Staphylococcus aureus, Salmonella, Escherichia coli, and Candida albicans which was harmful to human body. Results showed that water-soluble components of tea leaves mainly showed antibacterial activities for Staphylococcus aureus, Salmonella and Escherichia coli, and had the
strongest antibacterial effect on *Staphylococcus aureus*, with the lowest MIC of 0.03125 g/mL and lowest MBC of 0.25 g/mL. However, no effect was found on *Candida albicans*, with MIC and MBC were both higher than 3 g/mL. For fat-soluble components, it mainly showed antibacterial activities for *Candida albicans*, with the lowest MIC of 0.0625g/mL and lowest MBC of 0.25 g/mL. However, the antibacterial effect on *Staphylococcus aureus* and *Escherichia coli* was not significant, which MIC and MBC were both higher than 3 g/mL. The results would provide a theoretical basis for the development and application of dandelion tea and tea food.

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