In Vitro Antibacterial Effect and Sensitization Evaluation of Tea-Tree Oil Preparation

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Abstract. In order to promote the development and utilization of tea tree oil in China, the tea tree oil which was extracted from terpinen-4-ol typed Melaleuca alternifolia introduced and selected by Guangxi Forestry Science Institute, was tested in antibacterial activity, local irritation and sensitization research. The results demonstrated that the tea tree oil preparation to the selected staphylococcus aureus, epidermis staphylococcus aureus, escherich-ia coli, pseudomonas aeruginosa and candida albicans has good antibacterial activity. There were no irritating reactions observed in the dermal irritation test in rabbits and 0.5% tea tree oil has no sensitization in guinea pigs. This study confirms that tea tree oil has significant antibacterial activity, low -irritating and hypo-allergenic properties, promising a broad development value.

1. Introduction

Tea tree, also called as Melaleuca alternifolia, which originally originated in New South Wales and New Zealand. Tea tree oil is a colorless to yellowish essential oil extracted from the fresh leaves and twigs of Melaleuca alternifolia [1]. Tea tree oil is an essential oil developed rapidly in recent years, which has been proved to have broad spectrum antibacterial activity [2-3, 9]. It has been developed into preservatives, antibacterial agents and aromatic agents and other types of products used in cosmetics, drugs, food spices and other fields [4-5]. Tea tree oil contains more than 100 chemical components [6]. According to the main components of volatile oil, the biochemical types of tea tree oil can be divided into terpinen-4-ol type, 1,8-cineole type and intermediate type (i.e., terpinen-4-ol, 1, 8-cineole mixed type) [7-8]. It has shown that terpinen-4-ol is the main antibacterial component of tea tree oil [9-10]. Melaleuca alternifolia were first introduced in China in the 1990s [11], nearly 30 individual have been bred [12] which show significant different with the Australian origin in main composition content, aromaticity and antimicrobial activity [13-15], in this paper, the tea tree oil which was extracted from Guangxi Forestry Science Institute introducing and breeding of terpinen-4-ol typed Melaleuca alternifolia was adopted to tested in antibacterial activity, local irritation and sensitization research, in order to provide reference for the research and development of tea tree oil products in China.
2. Materials and methods

2.1. Materials and reagents
Terpinen-4-ol typed tea tree oil was provided by Guangxi Forestry Science Institute. Tea tree oil was prepared into tea tree oil sample with a volume fraction of 0.2%, 0.5% and 1% by using a sterile twin-80 solution with a volume fraction of 5%. Bacterial: staphylococcus aureus (26001), Staphylococcus epidermidis (26069), Pseudomonas aeruginosa (10211), E. coli (44104), Candida albicans (98001).

2.2. Experimental animals
Common grade white rabbit, body weight 1.5-2.3kg; Common grade guinea pig, weight 200-295g, adaptive feeding for one week.

2.3. Equipment
Agilent 7890A gas chromatograph (Agilent technologies, inc.), Autoclave mls-3020 (Panasonic Biomedical), Water-proof electric heating incubator GNP-9270 (Shanghai Jing Hong Laboratory Instrument Co., Ltd), Micro-vortex mixer wh-90a (Shanghai Zhen-Rong Factory), Electrothermal thermostatic incubator hhw21.cu600 (Shanghai Medical Instrument Factory).

2.4. Experimental methods

2.4.1. Qualitative and quantitative analysis of chemical constituents of tea tree oil. The quantitative analysis of tea tree oil was carried out by gas chromatography, and the main chemical components and contents were determined according to iso4730:2004 standard by referring to the qualitative analysis data of gas-mass coupling.

| Component       | content (%) | Range of ISO4730 |
|-----------------|-------------|------------------|
| α-pinene        | 1.66        | 1-6              |
| α-terpinene     | 10.09       | 5-13             |
| p-cymene        | 2.82        | 0.5-12.0         |
| 1, 8-cineole    | 1.36        | 0-15             |
| terpinen-4-ol   | 42.05       | ≥30              |
| α-terpineol     | 3.19        | 1.5-8            |

2.4.2. Determination of microbicidal actions (MIC). Tea tree oil sample solutions of 1% was diluted and then dissolved in nutrient AGAR and modified Martin AGAR medium respectively to prepare drug containing medium with different concentrations. After that, bacteria were seeded. MIC of tea tree oil was determined by bacterial growth. 1ml of each concentration of the drug solution to be tested was added to the sterile plate, and 9ml of hydrolyzed casein AGAR at 50-55℃ was immediately added, and rapidly mixed to form a series of plates with decreasing drug concentration. The final concentration of the drug in tablets was 1‰, 0.5‰, 0.25‰, 0.125‰, 0.0625‰, 0.03125‰. Add distilled water 1ml into the sterile plate, immediately add serum hydrolyzed casein AGAR 9ml at 50-55℃, mix to prepare the control. To each plate containing prepared sample, microbial suspension was
added $(1 \times 10^5 \text{ cfu/mL})$ and incubated for 24 hours at $37^\circ\text{C}$. The MIC of the drug was the lowest concentration that could inhibit the growth of the test bacteria.

2.4.3. Skin irritation test. Skin irritation test were performed as described in ISO 10993-10 (Biological evaluation of medical devices. Part 10: Tests for irritation and skin sensitization).

(1) Methods of animal pretreatment and drug administration

Twenty-four normal-grade white rabbits, weighing 1.5-2.3 kg, were randomly divided into 4 groups, namely, matrix control group and sample group (1%, 0.5%, 0.2% tea tree oil group, respectively), with half males and half females in each group. Long hairs on both sides of the back of rabbits were shaved off with a baby shaving machine, with an area of about $3\text{cm} \times 3\text{cm}$ on each side. A needle was used to cut the skin of the shed area on the right side of the rabbit's back in a "#" shape, with a small amount of blood oozing on the skin to make the damaged skin and the opposite side was normal intact skin.

The sample was evenly smeared for 0.2g on each side in sample group and the control group were applied with matrix.

Final score = (cumulative score of sample group)/(number of animals in sample group)−(cumulative score of control group)/(number of animals in control group).

(2) Evaluation provisions

Erythema: 0 points: no erythema; 1 point: mild erythema (visible to the naked eye); 2 points: moderate erythema (visible); 3 points: severe erythema; 4 points: severe erythema (purplish red erythema to mild eschar formation). Edema: 0 points: no edema; 1 point: slight edema (barely visible); 2 points: moderate edema (obvious swelling); 3 points: severe edema (skin uplift of about 1mm, clear contour); 4 points: severe edema (skin uplift >, 1 mm, extended range).

The score of skin irritation: Non irritation intensity: 0-0.49. Mild irritation: 0.5-1.99 points; Moderate irritation: 2.0-5.99; Severe irritation: 6.0-8.0.

2.4.4. Local sensitization test. Local sensitization test were performed as described in Technical Standard for Disinfection (2002) [16].

(1) Methods of animal pretreatment and drug administration

32 regular guinea pigs, half male and female, which weight 200-295g, divided into 4 groups randomly, which include of 2, 4-2 nitro chlorinated benzene group (positive control group), the matrix control group, 0.2% and 0.5% of tea tree oil group. The back of the guinea pigs were depilated with a range of about $3\text{cm} \times 3\text{cm}$. The sample was evenly smeared for 0.2g/a on the sample group and the matrix group respectively, and 0.5mL 1% 2, 4-2 nitro chlorinated benzene on the positive control group and keep for 6 hours. Repeat on the seventh day. After 14 days, the sample and matrix group were smeared with the same method, and the concentration of the 2, 4-dinitrochlorobenzene on the positive control group was changed to 0.1%. The allergic reactions of the skin were observed within 24h, 48h and 72h, and the allergic reactions were scored and sensitized.

(2) Evaluation provisions

The scoring criteria is the same as 2.4.3.2.

Incidence of sensitization = number of cases of animals with erythema or edema/total number of tested animals ×100%. Incidence of sensitization (%): 0-10: no sensitization; 11-30: mild sensitization; 31-60: moderate sensitization; 61-80: highly allergic; 81-100: extreme sensitization.

3. Results and Discussion

3.1. MIC test results

The results of the MIC test for tea tree oil preparations with different concentrations were shown in table 2. 1% of tea tree oil samples had antibacterial activity on staphylococcus aureus, staphylococcus epidermidis, Escherich-ia coli, Pseudomonas aeruginosa and candida albicans. The MIC values were 0.25‰, 0.25‰, 1‰, 1‰ and 1‰, respectively.
Table 2. Antibacterial activity of 1% tea tree oil sample.

| Drug dilutes concentration | AGAR eventually contains a drug concentration | Staphylococcus aureus | Staphylococcus epidermidis | Escherichia coli | Pseudomonas aeruginosa | Candida albicans |
|----------------------------|-----------------------------------------------|-----------------------|---------------------------|-----------------|-----------------------|-----------------|
| 1%                         | 1‰                                           | -                     | -                         | -               | -                     | -               |
| 0.5%                       | 0.5‰                                          | -                     | -                         | +               | +                     | +               |
| 0.25%                      | 0.25‰                                         | -                     | -                         | +               | +                     | +               |
| 0.125%                     | 0.125‰                                       | +                     | +                         | +               | +                     | +               |
| 0.0625%                    | 0.0625‰                                      | +                     | +                         | +               | +                     | +               |
| 0.03125%                   | 0.03125‰                                     | +                     | +                         | +               | +                     | +               |
| Distilled water 1 ml       | 1: 10                                         | +                     | +                         | +               | +                     | +               |

Note: (-) no colony growth, and (+) colony growth

3.2. Skin irritation test results

It shows that matrix and tea tree oil have no irritation to damaged and intact skin after 6 hours of contact in table 3-7. In the chronic dermal toxicity test, 1 hour and 24 hours after the end of the administration on day 7, the matrix and the tea tree oil group showed moderate irritation to damaged and intact skin. After 48 hours, the groups showed moderate irritation to intact and damaged skin except the high-dose tea tree oil group which showed mild irritation to intact skin. After 72 hours, both the intact and damaged skin of the matrix group presented moderate irritation, and the high-dose tea tree oil group presented moderate irritation to the damaged skin, and the other groups presented mild irritation to the intact and damaged skin. These results suggest that further improvement of the matrix is needed to avoid the influence of the matrix on the experiment.

Table 3. Acute skin irritation intensity score.

| group               | intact skin | damaged skin |
|---------------------|-------------|--------------|
| Stromal control     | 0           | 0            |
| 1% tea tree oil     | 0.17        | 0.17         |
| 0.5% tea tree oil   | 0.17        | 0.17         |
| 0.2% tea tree oil   | 0.17        | 0.17         |

Table 4. Intensity grading of acute skin irritation.

| group               | intact skin   | damaged skin |
|---------------------|---------------|--------------|
| Stromal control     | Non-irritating| Non-irritating|
| 1% tea tree oil     | Non-irritating| Non-irritating|
| 0.5% tea tree oil   | Non-irritating| Non-irritating|
| 0.2% tea tree oil   | Non-irritating| Non-irritating|

Table 5. Skin irritation intensity score at different time after chronic stimulation.

| group               | intact skin     | damaged skin   |
|---------------------|-----------------|----------------|
|                     | 1 h  | 24 h  | 48 h  | 72 h  | 1 h  | 24 h  | 48 h  | 72 h  |
| Stromal control     | 4    | 3.3   | 3.3   | 3.3   | 4    | 3.3   | 2.7   | 2     |
| 1% tea tree oil     | 2.3  | 2     | 1.3   | 1.3   | 3    | 2.7   | 2     | 2     |
| 0.5% tea tree oil   | 2.7  | 2     | 2     | 1.3   | 3.3  | 3.3   | 2     | 0.7   |
| 0.2% tea tree oil   | 3    | 3     | 2.3   | 1.3   | 3.2  | 3     | 2.5   | 1.7   |
Table 6. Classification of skin irritation intensity at different time after chronic stimulation on intact skin.

| group                  | 1 h     | 24 h    | 48 h    | 72 h    |
|------------------------|---------|---------|---------|---------|
| Stromal control        | Moderate irritation | Moderate irritation | Moderate irritation | Moderate irritation |
| 1% tea tree oil        | Moderate irritation | Moderate irritation | Moderate irritation | Mild irritation   |
| 0.5% tea tree oil      | Moderate irritation | Moderate irritation | Moderate irritation | Mild irritation   |
| 0.2% tea tree oil      | Moderate irritation | Moderate irritation | Moderate irritation | Mild irritation   |

Table 7. Classification of skin irritation intensity at different time after chronic stimulation on damaged skin.

| group                  | 1 h     | 24 h    | 48 h    | 72 h    |
|------------------------|---------|---------|---------|---------|
| Stromal control        | Moderate irritation | Moderate irritation | Moderate irritation | Moderate irritation |
| 1% tea tree oil        | Moderate irritation | Moderate irritation | Moderate irritation | Mild irritation   |
| 0.5% tea tree oil      | Moderate irritation | Moderate irritation | Moderate irritation | Mild irritation   |
| 0.2% tea tree oil      | Moderate irritation | Moderate irritation | Moderate irritation | Mild irritation   |

3.3. Local sensitization test

The matrix and tea tree oil show mild sensitization to guinea pig skin in the table 8-9, but 0.5% tea tree oil has no sensitization. The results suggest that the matrix has certain sensitization, and the tea tree oil with a content of 0.5% has activity of anti-sensitization.

Table 8. Score of allergic reactions.

| group                  | erythema | edema  |
|------------------------|----------|--------|
|                        | 24h | 48h | 72h | 24h | 48h | 72h |
| positive control       | 1.75 | 2.25 | 1.50 | 0.88 | 1.0 | 0.75 |
| Stromal control        | 0.50 | 0.50 | 0    | 0    | 0   | 0   |
| 0.5% Tea tree oil      | 0    | 0    | 0    | 0    | 0   | 0   |
| 0.2% Tea tree oil      | 0.25 | 0.25 | 0    | 0    | 0   | 0   |

Table 9. Skin sensitization evaluation.

| group                  | Sensitization rate | Sensitization evaluation |
|------------------------|--------------------|--------------------------|
| positive control       | 75%                | Highly                   |
| Stromal control        | 25%                | Mild                     |
| 0.5% Tea tree oil      | 0                  | non                      |
| 0.2% Tea tree oil      | 12.5%              | Mild                     |

4. Conclusion

This experiment showed that tea tree oil has good antibacterial activity to the staphylococcus aureus, epidermis staphylococcus aureus and candida albicans, MIC values to the staphylococcus aureus, epidermis staphylococcus aureus were 0.25‰, 1‰ to the Escherich-ia coli, Pseudomonas aeruginosa and candida albicans, and there were no irritating reactions observed in the dermal irritation test in rabbits and 0.5% tea tree oil has no sensitization in guinea pigs. In this paper, tea tree oil has been proved to have the advantages of antibacterial, low irritation and good biocompatibility, it is promising for use in food preservation and medical antibacterial products.

Acknowledgements

This work was supported by 2017 Science and Technology Major Project of Guangxi (No. AA17204058-21) and Science and Technology Project of Guangxi (No.AD18281083).
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