The in vitro antibacterial activity of a multiherbal combination used traditionally in Aden for topical treatment of Furuncles

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Abstract

Different amounts of the aqueous and methanolic extracts of *Lepidium sativum* seeds and *Flemingia grahamiana* glandular hairs of the pods as well as of their combination (multiherbal combination), that is used traditionally in Aden for topical treatment of furuncles, were tested for their antibacterial activity, by using a modified agar diffusion assay against *Staphylococcus aureus*(ATCC 29737),which is the most common bacteria causing furuncles. The aqueous extracts of *Lepidium sativum* seeds were found to possess antibacterial activity, while those of *Flemingia grahamiana* and multiherbal combination were found inactive. The ineffectiveness of the aqueous multiherbal combination may be due to a possible antagonistic interaction between its two components. The methanolic extracts only of *Flemingia grahamiana* and the multiherbal combination demonstrated moderately antibacterial activity. This result indicated that methanol is better than water in extracting the antibacterial active principles from *Flemingia grahamiana* that are responsible for the moderate antibacterial activity of the multiherbal combination against *S. aureus*.

Key word: Antibacterial activity, *Lepidium sativum*, *Flemingia grahamiana*, Multiherbal combination, seeds, glandular hairs.

Introduction

Traditional medicine (TM) is widespread throughout the world and has been practiced for centuries. Despite the development and spread of modern scientific medicine, TM is still dominant today, for examples, in Africa, up 80% of the population are using TM for primary health care (22). Moreover, in some countries, TM is an integral part of the formal health system as, for example China, the Democratic People's Republic of Korea, the Republic of Korea and Vietnam have fully integrated TM into their health care systems (18). Yemeni traditional medicine is still prevalent especially in the rural and urban areas. The wider acceptance of herbal medicine among Yemeni people is due to a number of reasons such as poverty, more accessibility, low cost of herbal drugs, shortage of hospitals and health centers in remote areas, as well as lack of faith to modern medicine. The Yemeni medicinal plants have been used by the indigenous people for the treatment of a number of skin diseases including furuncles (6,9,13,14). Furunculosis is a deep infection of the hair follicle leading to abscess formation with accumulation of pus and necrotic tissue. Furuncles appear as red, swollen, and tender nodules on hair-bearing parts of the body. Furunculosis tends to be recurrent and often spreads to family members either directly by skin contact or indirectly. The infection is most often caused by *Staphylococcus aureus* and resistance toward antimicrobials is an increasing problem. Methicillin resistant *S. aureus* is now endemic in many countries, and constitutes an emerging problem worldwide (15). In recent times, focus on plant researches has increased all over the world and a large body of evidence has been collected to show the immense potential of medicinal plants used in various traditional systems (16). Therefore, our work contributes in highlighting the role of the multiherbal combinations used in the traditional medicine in Aden for a topical treatment of furuncles. The selected multiherbal combination consists of a blend of *Lepidium sativum* seeds and *Flemingia grahamiana* glandular hairs of the pods. *Lepidium*

sativum L. (Family: Brassicaceae) is locally named Helf or Hab-Al Rashaad. The whole plant and seeds are used by the community of Yemen, Saudi Arabia and some other Arab countries as a good mediator for bone fracture healing (10). In addition, the seeds are used in Aden to treat inflammation of the prostate. In the traditional system of Indian medicine, cold infusions of seeds are used to relieve hiccough. The seeds are also used in chronic enlargement of liver and spleen and as carminative adjunct to purgatives. The bruised seeds, mixed with lime juice, are used as local application for the relief of inflammatory and rheumatic pains. The seeds are useful as poultices for sprains, and in leprosy, skin diseases, indigestion, dysentery, diarrhea, splenomegaly, asthma and restorative in general debility (17). *Flemingia grahamiana* Wight *&* Arn.*,* is one of the principal sources of the resinous powder known as 'Wars'. 'Wars' is a coarse purple or orange-brown powder consisting of the glandular hairs rubbed from the dry pods, principally used for dyeing silk; the active compound is called flemingin. In Yemen and other Arab countries, it is employed as a cosmetic, anthelmintic and a remedy for coughs and chills. In India, *Flemingia grahamiana* is used externally for skin diseases and internally as a purgative and specifically for colds (21). Although several scientific works have been done to evaluate the pharmacological activities including the antimicrobial activity of a number of medicinal plants used in Yemeni traditional medicine (1,3,4,5,7,8,12,19,20) only one study has been published to evaluate the antibacterial activity of a multiherbal combination used in herbal medicines in Yemen for the treatment of impetigo (2). Our work is the second scientific research to test the antibacterial activity of a multiherbal combination used in the traditional medicine in Aden for a topical treatment of furuncles.

Materials and Methods

Materials

Plant materials

Plant materials used in this study are *Lepidium sativum* L. (Brassicaceae = Cruciferae) seeds and *Flemingia grahamiana* Wight *&* Arn. (Fabaceae = Papilioanaceae), glandular hairs of the pods, as well as their combination in equal parts (multiherbal combination). The plant materials were purchased from local market in Aden.

Standard antibiotics

Susceptibility test discs of Amoxacillin 10µg and Erythromycin 15µg, used in the antibacterial assay as positive controls, were purchased from Himedia Laboratories Pvt. Ltd., (India).

Microorganism

Staphylococcus aureus (ATCC 29737) was used for determining the antibacterial activity of the tested plant materials..

Methods

Preparation of extracts

A blend, made of 30 g of the ground *Lepidium sativum* seeds and 30 g of *Flemingia grahamiana* glandular hairs of the pods, was extracted with water as used by herbal healer and designated as aqueous multiherbal combination. In addition, a methanolic extract of the above mentioned blend was prepared and designated as methanolic multiherbal combination. Moreover, two samples (methanol, and water extracts) were prepared from 30g of each ground plant material. Each of the plant materials was extracted 3 times (at ambient laboratory temperature, and under 4 hours shaking). The collected filtered methanolic and water extracts were concentrated to dryness, weighed and stored at $5C^{o}$ until used in the antibacterial activity test.

Antibacterial assay

Antimicrobial activity of the tested plant extracts was evaluated, using a modified agar diffusion assay (11,23). Different amounts of the extracts (5µl, 10µl, and 20µl equivalent to 2.5 mg, 5 mg, and 10 mg of the dried extracts respectively), applied on sterile paper discs (4 mm in diameter), were deposited on the surface of sterile agar (Mueller-Hinton agar) and inoculated with pure cultures of *Staphylococcus aureus*. Amoxacillin 10µg and Erythromycin 15µg discs were

placed on the same agar surface, served as positive controls. Discs impregnated with the corresponding solvents (methanol, and water), dried and placed on the agar surface, served as negative controls. Plates were then incubated for 24 h at 37°C. The inhibition of bacterial growth was determined by measuring the diameter of a clear zone around each disc and compared with the established inhibition zone size for individual antimicrobial agents. An average zone of inhibition was calculated for three replicates.

Statistics

Data are presented as mean ± standard deviation from three independent experiments.

Results

The yield of plant extracts

The percentage of the yield of each extract (w/w) was calculated as dry extract weight/dry starting material weight 100, (Table 1).

Table 1: The percentage of the yield of the tested plant extracts

Test Sample	Yield in %	
	Methanolic extract	**Aqueous extract**
Lepidium sativum seeds	8.3	5.7
Flemingia grahamiana glandular hairs of the pods	24	1.3
A blend of *Lepidium sativum* seeds and *Flemingia grahamiana* glandular hairs of the pods	22	5

Antibacterial activity of the tested plant materials

The results of the test for the antibacterial activity of the different extracts of *Lepidium sativum* seeds and *Flemingia grahamiana* glandular hairs of the pods and their combination against *Staphylococcus aureus* were presented in Table (2).

Table 2: Antibacterial activity of plant extracts against *Staphylococcus aureus*

Test sample	Amount of extract		
	2.5mg	**5mg**	**10mg**
	Diameter of inhibition zone in mm		
Lepidium sativum seeds			
Methanolic extract	0	0	0
Aqueous extract	12±1	14±0.74	16±1.2
Flemingia grahamiana glandular hairs of pods			
Methanolic extract	10±0.29	10±1	12±1.73
Aqueous extract	0	0	0
A blend of *Lepidium sativum* seeds and *Flemingia grahamiana* glandular hairs of pods			
Methanolic extract	10±1	12 ±0.29	12±1.73
Aqueous extract	0	0	0
Solvents (Methanol/ Water)	0		
Erythromycin 15µg	20		
Amoxacillin 10 µg	16		

The different amounts of the tested methanolic extracts of *Flemingia grahamiana* glandular hairs of the pods (2.5, 5mg and 10mg) showed almost similar moderate antibacterial activities with the inhibition zones (10, 10 and 12mm) compared to the positive control Amoxicillin (inhibition zone of 16mm) (Table 2, Fig. 1). The different amounts of the methanolic extracts of *Lepidium sativum* seeds were found ineffective (Table 2). However, the different amounts of the methanolic extracts of the multiherbal combination demonstrated antibacterial activities similar to those of the methanolic extracts of *Flemingia grahamiana* glandular hairs of the pods (Table 2, Fig. 1). The aqueous extracts (2.5, 5mg and 10mg) of *Lepidium sativum* seeds demonstrated antibacterial activities (inhibition zones: 12, 14 and 16mm respectively) approaching those of the positive control Amoxicillin (inhibition zone: 16mm) (Table 2, Fig. 1). On the other hand, the different amounts of the tested aqueous extracts of *Flemingia grahamiana* glandular hairs of the pods and of the multiherbal combination were found inactive (Table 2).

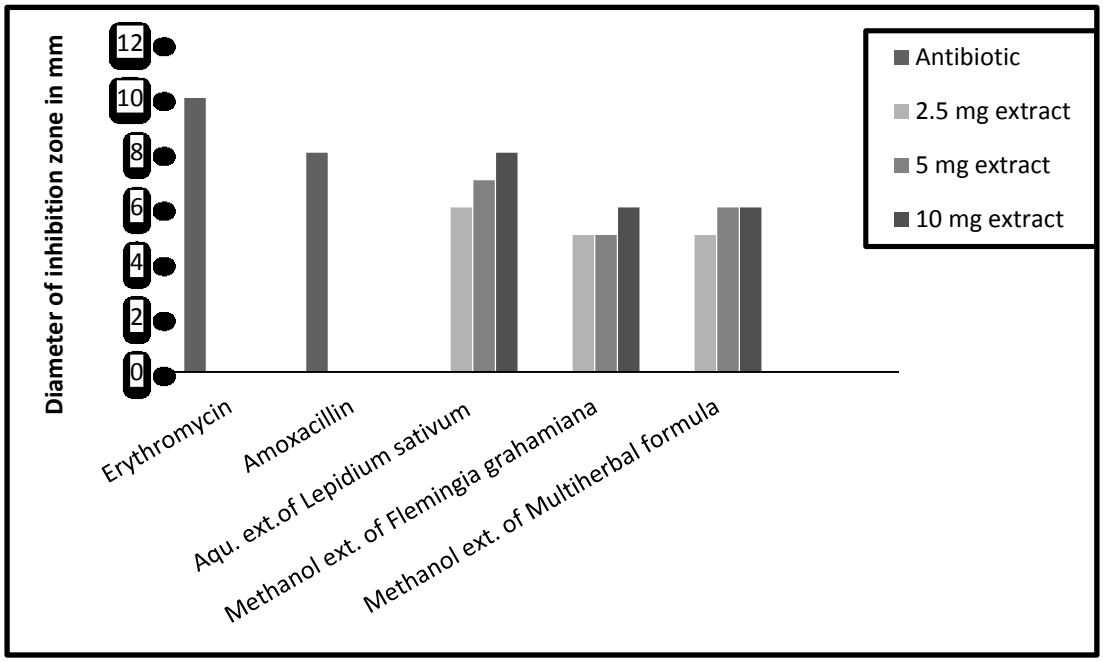

Fig 1 : Antibacterial activity of aqueous extract of Lepidium sativum and Methanolic extracts of Flemingia grahamiana and multiherbal formula Against *Staphylococcus aureus*.

Discussion

Aqueous and methanolic extracts of *Lepidium sativum* seeds, *Flemingia grahamiana* glandular hairs of the pods and a multiherbal combination, made of equal amounts of them, were investigated for their antibacterial activity against *Staphylococcus aureus*, the most common bacteria causing furuncles. The selection of the tested plant materials and their uses as an aqueous multiherbal combination is based on data collected from traditional herbal healers in Aden. In addition, the methanolic extracts of the multiherbal combination and their components were prepared and tested for their antibacterial activity against *S. aureus* for the purpose to evaluate the role of another solvent for releasing the antibacterial active constituents and consequently to examine the rationality of the traditional use of an aqueous multiherbal combination for the topical treatment of furuncles.

The different amounts of *Lepidium sativum* seeds aqueous extracts were found to possess antibacterial activity approaching that of the positive control Amoxicillin (Table 2, Fig. 1). The presence of antibacterial active constituents in the aqueous extract of *Lepidium sativum* seeds suggests that the seeds could be utilized for the treatment of infections caused by *S. aureus.* On the

other hand, all the tested amounts of the aqueous extracts of multiherbal combination and *Flemingia grahamiana* glandular hairs of the pods were found inactive against *S. aureus*. Although aqueous preparation of *Flemingia grahamiana* glandular hairs of the pods was highly appreciated and used in Yemen for skin diseases and cosmetic purposes, our present work and a previous study (5) showed that the aqueous extract of *Flemingia grahamiana* glandular hairs of the pods was inactive not only against *S. aureus* but also against other microorganisms (*Enterococcus faecalis*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Candida albicans*). The ineffectiveness of the aqueous extract of multiherbal combination could be explained by a possible antagonistic interaction between its two components. Another factor could include that the extracted active substances in the tested amount of the multiherbal combination was too low to produce inhibitory effect on the growth of *S. aureus*. The claimed usefulness of the aqueous multiherbal combination by herbal healer, for the treatment of furuncles may be due to the topical use of a large amount of the preparation. The moderate antibacterial activity, demonstrated by the different amounts of the tested methanolic extracts of multiherbal combination, is ascribed to the active constituents of the methanolic extracts of *Flemingia grahamiana* glandular hairs of the pods (Table 2, Fig. 1). Our work demonstrated the role of the extracting solvents, such as water that extracts the active constituents from *Lepidium sativum* seeds and methanol that releases the active constituents from *Flemingia grahamiana* glandular hairs of the pods, and also illustrated the irrational use of an aqueous extract of the multiherbal combination for the treatment of furuncles suggesting further phytochemical and pharmacological studies to evaluate the antibacterial effectiveness of multiherbal therapy used in Yemeni traditional medicine against a wide range of pathogenic microorganisms.

Conclusion

In conclusion, data obtained in this study illustrates the irrational practices in traditional medicine in Aden, by using an aqueous extract of a multiherbal combination, consisting of *Lepidium sativum* seeds and *Flemingia grahamiana* glandular hairs of the pods, for the treatment of furuncles. This study also demonstrates the importance of choosing the proper extracting solvents for releasing the active constituents that make the multiherbal therapy active against pathogenic microorganisms.

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اختبار الفعالية المضادة للبكتيريا لمركب عشبي يستخدم في الطب التقليدي في عدن كعلاج موضعي للدمامل

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الملخص

تم اختبار فعالية تراكيز مختلفة من مستخلصات الميثانول والماء لبذور الحلف Lepidium sativum والمادة الملونة (أحمر - برتقالي) والتي تسمى الورس هي عبارة عن شعيرات تغلف ثمار نبات الورس Flemingia grahamiana وأيضا للمركب العشبي المكون منهما والذي يستعمل في الطب التقليدي في عدن علاجاً للدمامل ضد بكتيريا *Staphylococcus aureus* المسببة الرئيسة للدمامل (furuncles) وذلك باستعمال طريقة (modified agar diffusion assay).أظهرت التراكيز المختلفة للمستخلصات المائية لبذور الحلف فعالية ضد البكتيريا في حين لم تظهر المستخلصات المائية لكل من الورس و المركب العشبي أي فعالية تذكر، مما يدل على احتمال حدوث تفاعلات مضادة بين مكوني المستخلص المائي للمركب العشبي أدت إلى اختفاء فعالية المستخلص المائي للحلف ومن ثم إلى عدم فعالية المستخلص المائي للمركب العشبي . أظهرت التراكيز المختلفة لمستخلصات الميثانول فقط لكل من الورس و المركب العشبي فعالية متوسطة مضاد للبكتيريا مما يدل على أنَ الميثانول مذيب فعال لاستخلاص المواد الفعالة من الورس وهي التي أكسبت مستخلص الميثانول للمركب العشبي فعالية ضد بكتيريا *Staphylococcus aureus*.

الكلمات المفتاحية: فعالية مضادة للبكتيريا، الحلف Lepidium sativum، نبات الورس Flemingia grahamiana ،مركب عديد الأعشاب ، بذور، شعيرات غدية.