

Antimicrobial Activity of Essential Oils of Some Yemeni Medicinal Plants

Rawiya H. Alasbahi¹ (Ph. D. / Pharmacy), Lyle E. Craker (Professor Ph. D. /Agronomy & Plant Genetics)
and Saida Safiyeva (MSc / Pharmacy)

Laboratories for Natural Products, Medicinal and Aromatic Plants, University of Massachusetts, Amherst, MA 01003-0910 USA

Abstract

Essential oils extracted from the Yemeni medicinal plants, *Acacia harala* Gifri & Thulin, sp. nov. aff. *ehrenbergiana* Hayne., bark; *Acalypha fruticosa* Forsk., leaves; *Capparis cartilaginea* Decne, leaves; *Indigofera sedgewickiana* Vatke & Hildebr., leaves and *Plectranthus cf. barbatus* leaves and stems were tested for antimicrobial activity against two gram-positive bacteria, *Staphylococcus aureus* (ATCC 25923) and *Enterococcus faecalis* (ATCC 19433), two gram-negative bacteria *Escherichia coli* (ATCC 25922) and *Pseudomonas aeruginosa* (ATCC 27853) and the yeast *Candida albicans* (ATCC 14053), using a qualitative agar diffusion test. All tested essential oils showed variable degrees of antimicrobial activity against one or more of the test microorganisms. Essential oils of *Acacia harala* and *Plectranthus cf. barbatus* were the most active

Introduction:

Essential oils play a role as potential antimicrobial agents for preservative purposes in food and cosmetic industries, for crop protection, and several medicinal applications (e.g. mouthwash, dental practice, aromatherapy). Antimicrobial, antifungal and antioxidant activities in essential oils have been reported in a number of studies (1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15). Moreover, the use of essential oils as antimicrobial agents could afford satisfaction, as they are generally inexpensive, biodegradable and non-toxic (16). Researches on the bioactivity of essential oils and their role in Yemeni traditional medicine are lacking. The present study was therefore undertaken to test the antimicrobial activity of essential oils extracted from some Yemeni medicinal plants, *Acacia harala* Gifri & Thulin sp. nov. aff. *ehrenbergiana* Hayne., bark (Fabaceae), a recently identified native Yemeni medicinal plant (17); *Acalypha fruticosa* Forsk., leaves (Euphorbiaceae);

Capparis cartilaginea Decne, leaves (Capparaceae); *Indigofera sedgewickiana* Vatke & Hildebr., leaves (Fabaceae) and *Plectranthus cf. barbatus* leaves and stems (Labiatae). Determination of the antimicrobial activity of a number of extracts of different polarities prepared from the above-mentioned plant tissues has been reported in our previous work (18). However, screening their essential oils for antimicrobial activity was carried out to assess the role of essential oils in the overall antimicrobial activity of the tested plants, and to help providing scientific justification for the utilization of these plants in traditional medicine.

Materials and Methods

Plant Materials

The plants used in this study, bark of *Acacia harala*, leaves of *Acalypha fruticosa*, leaves of *Capparis cartilaginea*, leaves of *Indigofera sedgewickiana* and leaves and stems of *Plectranthus cf. barbatus* were collected in Abyan, Taiz, and Yaffee Heights, Republic of Yemen. The first four

Corresponding author: Rawiya Alasbahi, e-mail: raalasbahi@yahoo.com

plants were authenticated by Dr. Al-Gifri, A. N., Department of Biology, University of Aden, Yemen, and Dr. Al khulaidi A.-W., Ministry of Agriculture, Yemen. The last one was authenticated by Dr. Mats Thulin, Department of Systematic Botany, Evolutionary Biology Centre, Uppsala University, Sweden. The leaves, bark and stems were shade dried at ambient temperature (30 °C) and ground individually in a Wiley mill (20 mesh).

Essential oils extraction Essential oils were extracted from the plant material (100-150g) by using a distillation-solvent extraction head of the type described by Lickens and Nickerson (19). A sample flask was charged with powdered plant material in distilled water, the solvent flask contains pentane. The distillation time was three hours. The essential oil was obtained after the removal of pentane by using a gentle stream of N₂ at room temperature.

Test microorganisms

Two gram-positive bacteria *Staphylococcus aureus* (ATCC 25923) and *Enterococcus faecalis* (ATCC 19433) and two gram-negative bacteria *Escherichia coli* (ATCC 25922) and *Pseudomonas aeruginosa* (ATCC 27853) and the yeast *Candida albicans* (ATCC 14053) were used as test microorganisms. An inoculating loop full of each bacterial and yeast culture was inoculated on individual tube slant of tryptic soy agar (for bacteria) and potato dextrose agar (for the yeast) for overnight incubation at 37°C. A suspension of each bacteria and yeast from the 24h Slant was prepared in sterile saline solution to obtain turbidity equal to a Mc Farland standard 0.5 (108CFU/ml).

Antimicrobial assay

Antimicrobial activity of essential oils was evaluated using an agar diffusion test (20, 21). The prepared suspension of each microorganism was streaked on the surface of Muller-Hinton agar contained in Petri plates (9 cm in diameter). A sterile paper disc (0.6 cm in diameter) impregnated with 3 µl of undiluted essential oil was placed on the agar surface. An antibiotic-containing paper disc (Methicillin 5µg, Polymyxin B 300 units,

Vancomycin 30 µg or Nystatin 100 units, depending on the microorganism) served as a positive control was placed on the same agar surface. The third paper disc impregnated with 3 µl of sterile distilled water, dried and placed on the same agar surface, served as a negative control. The plates with paper discs were inverted and incubated for 24h at 37°C. The inhibition of microbial growth was determined by measuring the diameter of the clear zone around each disc at the end of the incubation time. An average zone of inhibition was calculated for three replications.

Statistics

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

Results

The distillation-extraction of *A. harala*, bark; *A. fruticosa*, leaves; *C. cartilaginea*, leaves; *I. sedgewickiana*, leaves and *P. cf. barbatus* leaves and stems, gave essential oils in the yield of 0.14%; 0.3% ; 0.1% ; 0.22 %, 0.24% and 0.17% based on dried plant material respectively.

The essential oils from *A. harala* bark and *P. cf. barbatus* leaves and stems showed antimicrobial activity against almost all the tested microorganisms (Table 1). An inhibition of the growth of *C. albicans* and *S. aureus* exhibited by the essential oils of *A. harala* bark and of *P. cf. barbatus* leaves and stems was almost nearly equal to the inhibition produced by the positive controls (Figures 1 and 2). Among the tested essential oils, only that of *A. harala* bark was found able to produce a notable inhibition of *E. coli* growth (Figure 3).

The essential oils of the leaves of *A. fruticosa*, *C. cartilaginea* showed some antimicrobial activity against one of more of the tested gram-positive bacteria, as well as a moderate activity against *C. albicans*. None of these essential oils were active against the tested gram-negative bacteria. *I. sedgewickiana* essential oil was found able to inhibit only the growth of *S. aureus* (Table 1).

Discussion

The present study reports the antimicrobial screening of

essential oils extracted from five Yemeni medicinal plants used in traditional medicine in Yemen for a variety of diseases. *A. harala* bark, is used by indigenous people externally to treat back pain, *A. fruticosa* leaves are commonly used externally to treat skin diseases (22), *C. cartilaginea* leaves are used for the treatment of snake bites (22), tumors, furuncles, lung diseases, dental pain and rhinitis and *P. cf. barbatus* leaves, are used externally as homeostatic agent, and internally in the form of decoction to reduce bleeding in heavy menses. Although some of the medicinal plants tested in this study are not used in traditional medicine in Yemen for the treatment of infectious diseases, related species were reported to be useful in other countries (23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33). Because no essential oil could be extracted from *I. sedgewickiana* pods, which are eaten to relieve kidney pain, we investigated the antimicrobial activity of the essential oil extracted from the leaves of this plant with the intention of revealing the role of the essential oil in the antimicrobial activity of the leaves, which usually are not used in Yemeni traditional medicine.

An interesting result was the inhibition of the growth of *C. albicans* and *E. coli* by the essential oil of *A. harala* bark (Table 1). The level of microbial inhibition of *C. albicans* was nearly equivalent to that of the antibiotic nystatin (Figure 2). Essential oil of *A. harala* bark was also found to produce a notable inhibition of the growth of *S. aureus* (Figure 1). The recently identified Yemeni *A. harala* was first investigated by us. In our previous work (18), the aqueous extract of *A. harala* bark was found to exhibit antimicrobial activity against all tested microorganisms except *E. coli*. Consequently, a number of plant constituents (polar and non-polar) may be involved in the antimicrobial activity of *A. harala* bark. Gas chromatography-mass spectrometry (GC-MS) analysis of *A. harala* bark essential oil revealed the presence of a number of compounds such as 2-carboxymethyl-3-n-hexylmaleic acid anhydride, patchoulene, α -cedrene and trans-8-methyl-1- β -acetyl-hydrindane (our personal data),

which could be involved in the antimicrobial activity of *A. harala* bark. Barks of other *Acacia* species have also been found to possess antimicrobial activity (23, 27, 34, 35, 36, 37, 38).

This is the first report about the antimicrobial activity of the essential oil of *P. cf. barbatus* leaves and stems, which were found active against all tested microorganisms especially gram-positive bacteria and *C. albicans*. The microbial growth inhibitory effects of the essential oils of *P. cf. barbatus* leaves and stems against *S. aureus*, *E. faecalis*, and *C. albicans* were nearly approaching those of the positive controls (Table 1, Figures 1, 2). A number of components were identified in the essential oils of the leaves (e.g. α -cadinene, α -copaene, trans-caryophyllene, eremophilene) and of the stems (such as Δ -cadinol, elemol, ent-spathulenol, β -selinene, 7(11)-selinen-4 α -ol) of *P. cf. barbatus* by using GC/MS analysis (our personal data). Moreover, different extracts of different polarities of the same plant tissues were found active mostly against *S. aureus*, *E. faecalis*, and *C. albicans* (18). Thus a number of other constituents beside those contained in the essential oil may be responsible for the antimicrobial activity of *P. cf. barbatus* leaves and stems. Antimicrobial principles have also been reported in other *Plectranthus* species (26, 30, 39, 40).

The essential oil of the *A. fruticosa* leaves showed some antimicrobial activity against tested gram positive bacteria and *C. albicans* (Table 1). In our previous work (18), chloroform and hexane extracts of the leaves of the same plant were found to exhibit antimicrobial activity against all tested microorganisms. Therefore, the partial role of the essential oil in the antimicrobial activity of *A. fruticosa* leaves provides an additional scientific justification for the use of this plant in the Yemeni traditional medicine for the treatment of skin diseases. GC/MS analysis of the essential oil of the leaves of this plant indicated the presence of a number of components such as caryophyllene, α -humulene, α -copaene, 4-terpineol, and γ -cadinene (our personal data). These compounds may

contribute in the antibacterial activity of the leaves of this plant. In addition, previous studies reported the antibacterial activity of *A. fruticosa* leaves used in folk medicine in Tanzania (41) and India (42).

The essential oil of *C. cartilaginea* leaves was found able to inhibit the growth of *C. albicans* (Table 1). Out of different extracts with different polarities of *C. cartilaginea* leaves tested for their antimicrobial activity against the same microorganisms used in this study (18), only methanol extract was found active, and only against

S. aureus. Thus, different types of compounds may be contained in the leaves and are responsible for the antimicrobial activity. GC/MS analysis of the essential oil *C. cartilaginea* leaves revealed the presence of a number of compounds such as (+)-carvotanacetone, copaene, geranyl acetone, β -cadinene (our personal data), which could play a role in the antifungal activity of the essential oil of the leaves.

The essential oil of the leaves of *I. sedgewickiana* showed some activity against

Table 1: Antimicrobial activity of essential oils of some Yemeni medicinal plants

Essential oil	Test microorganisms				
	<i>S. aureus</i>	<i>E. faecalis</i>	<i>E. coli</i>	<i>P. aeruginosa</i>	<i>C. albicans</i>
Diameter of inhibition zone in mm (\pm Standard deviation)					
<i>Acacia harala</i> Bark	15 (± 1)	n.t.1	12 (± 0.6)	n.t.1	25 (± 1.2)
<i>Acalypha fruticosa</i> Leaves	8 (± 1)	10 (± 0.6)	0	0	15 (± 1.5)
<i>Capparis cartilaginea</i> Leaves	0	9 (± 1)	0	0	18 (± 1.2)
<i>Indigofera sedgewickiana</i> Leaves	11 (± 1)	n.t.1	0	n.t.1	0
<i>Plectranthus cf barbatus</i> Leaves Stems	20 (± 1.5) 20 (± 1.2)	15 (± 1.7) 15 (± 1.5)	8 (± 0.6) 7 (± 0.6)	7 (± 0.6) 0	15 (± 1) 20 (± 1)
Positive control2					
Antibiotic 1	23 (± 0.6)	19 (± 0.6)	15 (± 0.6)	15 (± 1)	27 (± 0.6)
Antibiotic 2	19 (± 1)	—	—	—	—
Negative control					
Distilled water	0	0	0	0	0

1n.t.: not tested.

2Positive control: For *S. aureus*: antibiotic 1: Methicillin 5 μ g; antibiotic 2: Vancomycin 30 μ g

For *E. faecalis*: antibiotic 1: Vancomycin 30 μ g

For *E. coli*: antibiotic 1: Polymyxin B 300 units

For *P. aeruginosa*: antibiotic 1: Polymyxin B 300 units

For *C. albicans*: antibiotic 1: Nystatin 100 units

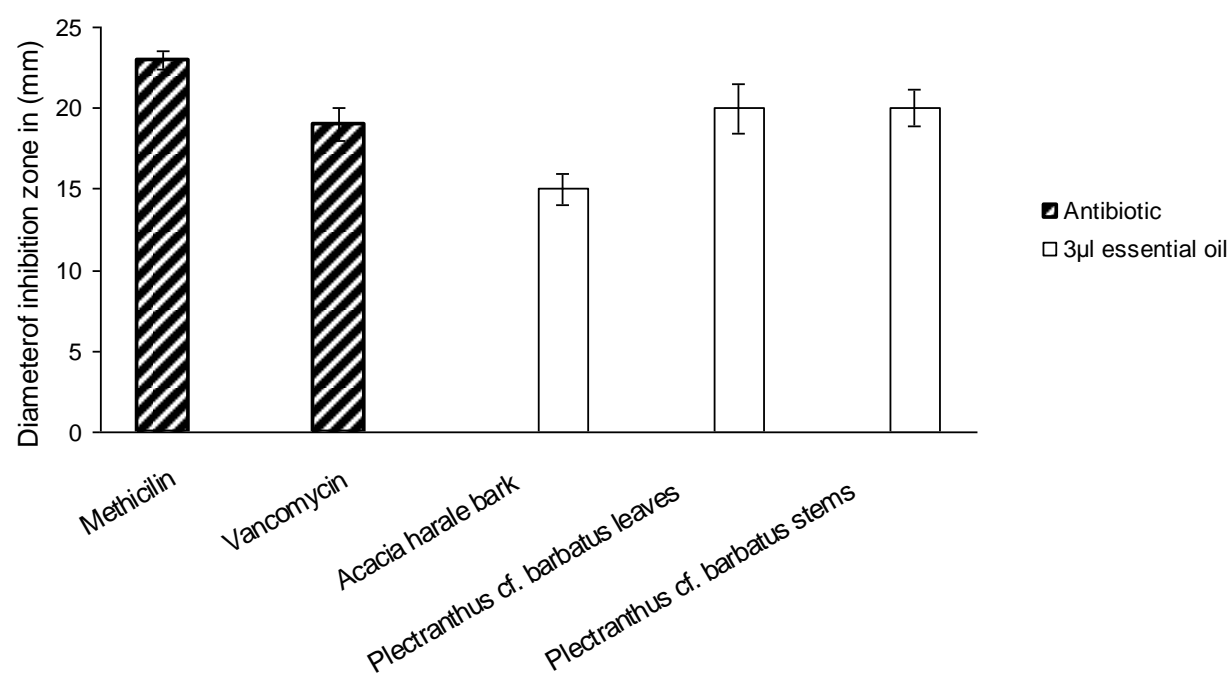


Figure 1: Antibacterial activity of the essential oils of *Acacia harale* bark and *Plectranthus* cf. *barbatus* leaves and stems against *Staphylococcus aureus*

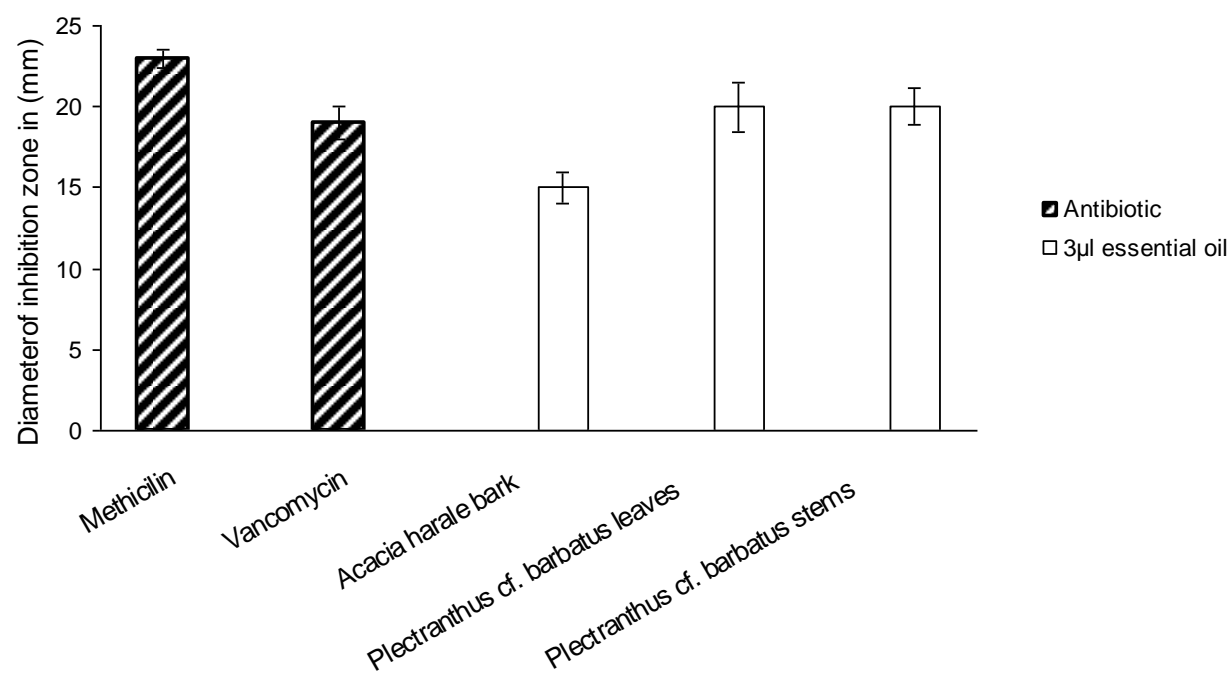


Figure 1: Antibacterial activity of the essential oils of *Acacia harale* bark and *Plectranthus* cf. *barbatus* leaves and stems against *Staphylococcus aureus*

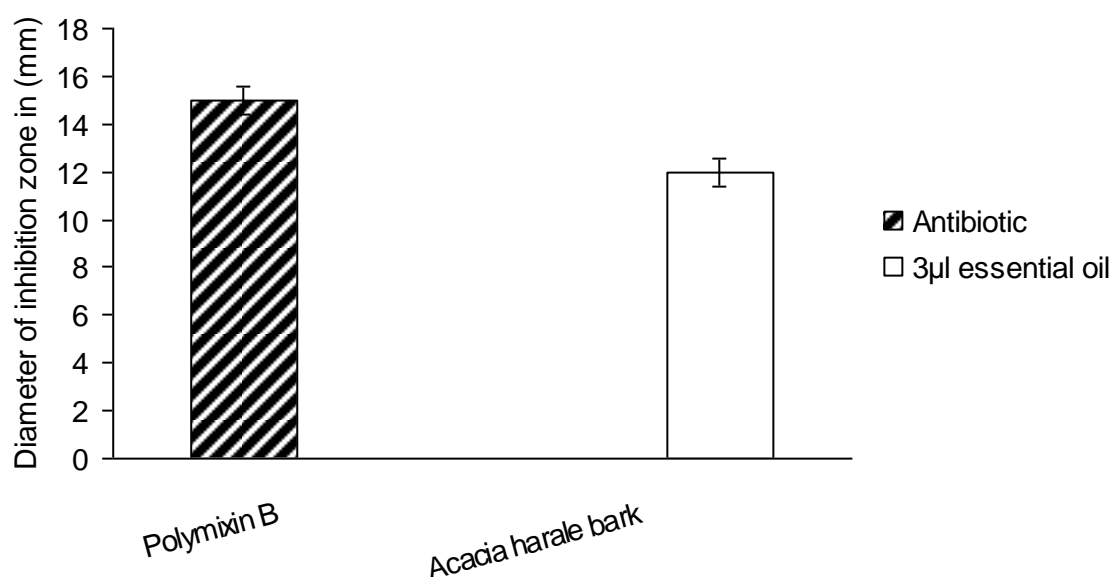


Figure 3: Antibacterial activity of the essential oil of *Acacia harale* bark against *Escherichia coli*

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