Agric. Chem. Biotechnol. **48**(1), 29-31 (2005)

Article

Antimicrobial Activity of the Extracts of *Forsythia suspensa* and *Dendranthema indicum*

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Received September 30, 2004; Accepted February 23, 2005

Antimicrobial activities of extracts of *Forsythia suspensa* fruits and *Dendranthema indicum* buds and flowers against bacteria; *Escherichia coli, Staphyloccus aureus,* and *Bacillus subtilis,* and fungi; *Aspergillus flavusn, Rhizopus stolonifer, Penicillium citrinum, Aspergillus niger,* and *Saccharomyces carlsbergensis,* were investigated. The plants were extracted with 70% ethanol and the extracts were used for antimicrobial activity assay. All extracts exhibited significant inhibition activity against microorganisms at concentrations ranged from 1.66 to 100 μl/m*l*. The inhibition activity by the extract of *D. indicum* buds was stronger than by the extract of *F. suspensa* fruits and *D. indicum* flowers. *D. indicum* buds showed antimicrobial activity against *S. aureus* which was comparable to other medicinal plants. *F. suspensa* fruits and *D. indicum* flowers was suggested to be valuable sources as antimicrobial ingredients in food industry.

Key words: *Antimicrobial activity,* Forsythia suspensa, Dendranthema indicum

Antimicrobial activities of various medicinal plants and derivatives have been reported by many workers. A number of synthetic chemicals have been suggested to convert ingested materials into toxic substances or carcinogens by increasing the activity of microsomal enzymes[1)]. Some chemicals require caution in handling because they are corrosive and their vapors can irritate the eyes and respiratory tract. In contrast, herbs and their derivatives and decoctions possessing antimicrobial activity have been known to have beneficial effects, showing no health problems to the handler and consumer.

Forsythia suspensa is a climbing plant widely distributed in south eastern Asia. The dried fruit extracts have long been used in the Chinese and Japanese folk medicines to treat gonorrhea, erysipelas, inflammation, pharyngitis, pyrexia, tonsillitis, and ulcer. They have also potential antibacterial effects[2,3)]. In China, the dried buds and flowers of *Dendranthema indicum* have been used as popular tea due to their good smell. In folk medicine it was used as an antipyretic, to clear the eye and the mind, and as an antitoxin. It is widely used as a remedy for the common cold, headache, dizziness, red eye, swelling, and hypertension.

This work was performed to evaluate the antimicrobial potential of medical plants. Among the more than ten medical plants, *F. suspensa* and *D. indicum* were selected to evaluate for the activity against eight microorganisms including three bacteria and five fungi.

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Abbreviations: PDB, Potato Dextrose Broth; CFU, colony-forming units; PDA, Potato Dextrose Agar; IZD, inhibition zone diameter; MIC, minimal inhibitory concentrate

Materials and Methods

Plant material. *F. suspensa* and *D. indicum* used were obtained from Zhejiang, China, and they were dried at room temperature.

Plant extraction. The samples (150 g) were extracted with 70% ethanol (v/v) using a percolator apparatus[4)]. The extracts were filtered and dried under reduced pressure at 40°C. The volume of the extracts were adjusted to 150 m*l* with 70% ethanol (v/v).

Microbial strains. Microorganisms frequently reported in food spoilage were supplied by the microbiology laboratory of Zhejiang Forestry University. Three species of bacteria, *Escherichia coli*, *Staphyloccus aureus*, and *Bacillus subtilis*, and five species of fungi, *Aspergillus flavusn*, *Rhizopus stolonifer*, *Penicillium citrinum*, *Aspergillus niger*, and *Saccharomyces carlsbergensis* were used in this study.

Preparation of inoculum. Bacteria inocula were prepared by growing cells in nutrient broth at 37°C for 24 h. Fungi were grown in Potato Dextrose Broth (PDB) at 28°C for 48 h. Cells were diluted with saline solution to provide initial cell numbers of about 10^5-10^6 CFU · ml^{-1} [5)]. An aliquot of 1 m*l* is used for antimicrobial assay.

Antimicrobial activity assay. The antibacterial activity of the extracts was determined by the disc diffusion test[6)]. The

plates containing Nutrient Agar or Potato Dextrose Agar (PDA) medium were used. An aliquot of 1 m*l* was evenly spread on agar using a glass rod spreader. The plates were left at room temperature for 1 h to allow the agar surface to dry. The extracts were added at 25 μ*l* per 5 mm diameter filter paper disc and placed on the culture medium. Ethanol was added at 25 μl/disc to provide a negative control. Bacteria and fungi were incubated at 37 and 28°C, respectively, for 7 days. At the end of the incubation the antimicrobial activity was evaluated by measuring the inhibition zone diameter (IZD). An inhibition zone of 14 mm or greater (including diameter of the disc) was considered as high antimicrobial activity[7].

Minimal inhibitory concentrate (MIC) determination. Serial two-fold dilutions of each extract were added to sterile molten culture medium at the appropriate volume to produce the concentration range of 1.66-100 μ$l \cdot ml^{-1}$. Control dishes contained equal volume of ethanol. After cooling and drying, the plates were inoculated in spots of 0.1 m*l* with each microorganism cell suspension (10^4 CFU $\cdot ml^{-1}$), and bacteria and fungi were incubated at 37 and 28°C, respectively, for 48 h. MIC values were determined as the lowest concentration of the extracts where the absence of growth was recorded.

Results and Discussion

Antimicrobial activity of the extracts. *F. suspensa* and *D. indicum* extracts exhibited different inhibition levels against microorganisms (Table 1). Extract of *D. indicum* buds showed stronger inhibition (5.8 ± 0.2 mm to 21.9 ± 1.1 mm) than those of *F. suspense* fruits and *D. indicum* flowers, with highest inhibition against *S. aureus* (21.9 ± 1.1 mm) and *B. subtilis* (16.6 ± 0.7 mm) and weak inhibition against *P. citrinum* and *E. coli*. Extract of *D. indicum* flowers (6.1 ± 0.4 mm to 15.1 ± 0.7 mm) had antimicrobial activity against 7 out of the 8 (87.5%) test microorganism strains, showing the highest inhibition against *B. subtilis* (17.4 ± 1.3 mm) and *S. aureus* (15.1 ± 0.7 mm) and no inhibition against *R. stolonifer*. Extract of *F. suspense* fruits showed low inhibition (5.7 ± 0.6 mm to 9.5 ± 0.4 mm) against most of the microorganisms tested, and also did not inhibit the growth of *R. stolonifer*, too.

After 7 days incubation, all extracts showed inhibition activity against three bacteria strains, but no inhibition against fungi; in particular, the extract of *D. indicum* bud showed the inhibition diameter zone at 9.0 ± 0.8 mm to18.1 ± 1.2 mm. Although the extract of *F. suspense* showed the lowest inhibition activity after 48 h, it was stronger than that of *D. indicum* flowers after 7 days.

MIC of extracts. MIC values of the extracts of *F. suspensa* and *D. indicum* are shown in Table 2. Sensitivity of *D. indicum* buds extract was two- to four-fold higher than those of *F. suspensa* fruits and *D. indicum* flowers extracts. The antimicrobial activities of the extracts were higher against

Table 1. Antimicrobial activities of extracts of *F. suspensa* and *D. indicum* after 48 h-incubation

Microorganisms	Inhibition diameter zone (mm)			
	F. suspensa fruits	*D. indicum* buds	*D. indicum* flowers	Ethanol (control)
E. coli	7.9 ± 0.5[a]	9.5 ± 0.7	8.0 ± 0.8	-[b]
B. subtilis	9.5 ± 0.4	16.6 ± 0.7	17.4 ± 1.3	-
S. aureus	7.8 ± 0.6	21.9 ± 1.1	15.1 ± 0.7	-
S. carlsbergensis	5.7 ± 0.6	11.5 ± 0.6	6.5 ± 0.4	-
P. citrinum	6.1 ± 0.2	5.8 ± 0.2	6.1 ± 0.4	-
R. stolonifer	-	11.5 ± 0.7	-	-
A. niger	9.9 ± 0.6	12.3 ± 0.6	9.8 ± 0.7	-
A. flavusn	6.9 ± 0.7	14.6 ± 0.7	9.8 ± 0.9	-

[a]Data are means ± S.D, 9 replicates.
[b]-: No inhibition.

Table 2. Minimal inhibitory concentration (MIC) of the extracts

Microorganisms	μl/m*l*		
	F. suspensa fruits	*D. indicum* buds	*D. indicum* flowers
E. coli	3.13	1.66	3.13
B. subtilis	12.5	3.13	12.5
S. aureus	3.13	1.66	6.25
S. carlsbergensis	50.0	6.25	100.0
P. citrinum	-[a]	50.0	-
R. stolonifer	-	25.0	-
A. niger	100.0	50.0	50.0
A. flavusn	50.0	6.25	25.0

[a]-: No inhibition.

bacteria that against fungi.

Several studies have reported that many medicinal plants, such as *Landolphia owerrience*[8], *Anthocleista djalonensis, Nauclea latifolia, Uvaria afzalii*[9], *Memecylon malabaricum*[10], *Artemisia* species[11], and other twenty-five medicinal plants of the island Soqotra[7] had antimicrobial activities. In this study, *D. indicum* buds showed antimicrobial activity against *S. aureus* (21.9 ± 1.1 mm), which was comparable to other medicinal plants. Similar to *D. indicum* buds, *D. indicum* flowers and *F. suspensa* fruits have medium antimicrobial activities. Taken from experimental data, *F. suspensa* and *D. indicum* were suggested to be valuable sources as antimicrobial ingredients in food industry.

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