Antimicrobial Activity of Some Edible Plants: Lotus (Nelumbo nucifera), Coffee, and Others

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ABSTRACT

A number of edible plant species were investigated for antifungal agents. Whole sprouts and extracts of plant organs were tested in several assays, including bioautography. Amaranth, coffee (Coffea arabica), rice, coleus, violet, chervil, and lotus (Nelumbo nucifera) showed antifungal activity. Rhizomes of lotus had potent antifungal activity against Aspergillus niger, Trichoderma viride, and Penicillium spp. Further work is merited for characterization of this antifungal agent. Screening of sprouting plants and terrestrial aquatic plants may be a fruitful approach to finding new antimicrobials.

A major need of the food industry is food preservation. The consumer has a preference for “natural” foods. Some food preservatives, such as benzoic acid (8) and antibiotics, fall into this natural category but are still considered chemicals. Others, such as spices and essential oils (5,6,10), are suitable for special applications but are not used in general, because they change the flavor of foods.

The goal of the present study was the detection of antimicrobial agents in edible plants. It is probable that an antimicrobial from a plant with a history of edibility would have an enhanced chance of low toxicity and eventual regulatory approval. We focused on sprouts, because it seemed likely that tender, emerging seedlings need resistance factors against microbial attack, and this may be a likely stage to find useful preservatives. We had observed that spraying barley does not mold, while wheat and soybean could not be sprouted without copious mold formation. Barley produces the antimicrobials hordatine (14) and chitinase (13). Also, with coffee beans, we had observed that abundant mycelium present on the bean macroscopically disappears, once the sprout emerges (Haas unpublished, 1985). Baumann has shown that during sprouting there is an increase in caffeine (1-4). We report experiments here that suggest the presence of an additional antifungal, other than caffeine (9), in coffee sprouts.

While most of our reported work is on sprouts, the study was expanded to other plant organs of lotus, the Asiatic Holy Lotus, which can grow in microbially rich waters. The seeds and rhizomes of this plant are eaten widely throughout the Orient, and the rhizomes exported. We report that both organs have antifungal activities.

MATERIALS AND METHODS

Microbes and media

Microbes used in this study were Staphyloccocus epidermidis ATCC 12228, Escherichia coli strain DH10B (Bethesda Research Laboratories), Candida utilis NRRL Y-900 and Saccharomyces cerevisiae NRRL Y-2034, Penicillium camemberti, and Penicillium chrysogenum from Kraft General Foods, and Trichoderma viride from Dr. M. Mandels, United States Quartermaster Laboratories. A caffeine resistant strain of Aspergillus niger was obtained from Dr. B. Doonan, Kraft General Foods, and was maintained on Czapek pH 6 agar (Difco) supplemented with 5 mg/ml caffeine. Other fungi were maintained and tested on Czapek pH 6 agar; bacterial cultures were maintained and tested on Luria-Bertani pH 7.4 agar (Difco).

Plants

Viable seeds of amaranthus, anise, asparagus, caraway, chamomile, chervil, coleus, fennel, forget-me-not, leek, parsley, radish, water cress, and violets were purchased from Burpee Seeds Co., Warminster, PA. These seeds had not been treated with antifungals. Viable Coffea arabica beans and viable rice, barley, and wheat seeds (caroposes) were supplied by Kraft General Foods. Lotus (Nelumbo nucifera) seeds were obtained from Weerachia Nanakorn of the New York Botanical Garden from a market in Thailand, and lotus rhizomes were purchased at a produce market in Chinatown, New York.

Growth, extraction, and screening of sprouts

With the exception of coffee and lotus, seeds were germinated on Whatman No. 1 filter paper moistened with glass distilled water and used 2 to 3 d after germination. Coffee beans were surface sterilized by rinsing briefly in a 1:1 mixture of 95% ethanol and Chlorox bleach, followed by rinsing in three changes of 2 L of sterile glass distilled water for 4 h each change, followed by overnight imbibition in sterile glass distilled water. Coffee was sprouted in sterile culture containers on moistened filter paper and...
2- to 3-week-old sprouts examined similarly to other sprouts. Lotus was sprouted under water for 3 to 5 d.

Whole sprouts were laid flat on top of 30 ml of microbial media (see Microbes and Media) in 100-mm plastic petri dishes. Other sprout tissue was frozen in liquid nitrogen, freeze-dried, and ground in a mortar and pestle. Approximately 0.1 g was placed on plates preincubated with a microbial test organism. Preincubated plates were prepared as follows: conidia from 5-d-old 5 ml Czapek agar slants in 13 × 100-mm tubes were suspended in 0.001% vol/vol Tween 20. One milliliter of spore suspension was used to inoculate 500 ml of media at 45°C.

For further evaluation some sprouts, with roots and cotyledons removed, were macerated with a mortar and pestle at room temperature in 10 volumes per weight of 4:1 methanol:water, and centrifuged for 10 min at 10000 × g. A portion of this supernatant was fractionated into classes of compounds according to polarity, essentially by the method outlined by Harborne (7). Crude methanol extract and fractions were stored at -20°C until tested. Methanol-water extracts and neutral, basic, moderately polar, and polar extracts were dried, and the residues suspended in 1/10 original extraction volumes of sterile water and, subsequently, tested for antimicrobial activity by application to Penicylinders (Fisher Scientific) or wells 6 mm deep and 6 mm in diameter cut into inoculated agar plates. Zones of no growth, or inhibition of growth, or inhibition of conidiation around sprout tissue, Penicylinders, or wells were considered positive for an antimicrobial effect. Potassium sorbate was used as positive control and residues from dried extraction solvents as negative controls.

Bioautography (12)

Extracts were further fractionated by thin-layer chromatography (TLC) on Baker 250-μm silica plates, developed with 1:1 ethyl acetate:ethyl ether (for lotus) or 90:9:1 methanol:water:glacial acetic acid (for coffee). TLC plates were overlayed with a thin layer of 45°C Czapek medium containing 7 g/L agar preincubated with 0.5 ml of spore suspension per 100 ml media. For lotus rhizome, areas corresponding to zones of inhibition were excised from a duplicate uninoculated TLC plate, and the active fraction was eluted with 90:10 methanol:water. This fraction was chromatographed in the first dimension in 90:9:1 methanol:water:acetic acid and the second dimension in 90:9:1 methanol:water:ammonium hydroxide. The active fraction was eluted from the silica of a duplicate TLC plate and scanned between 200 and 1100 nm wavelength in a Perkin-Elmer Lambda 2 UV/VIS Spectrophotometer.

RESULTS

Activities of whole tissues and extracts

Table 1 shows activities of whole sprouts tissues. Table 2 shows activities of extracts.

Bioautographies

The most striking zones of inhibition were seen for a basic extract from rhizomes of lotus against A. niger (3.1 cm/g fresh rhizome tissue) and T. viride (1.1 cm/g). T. viride is more sensitive to this antifungal activity than is A. niger. The rhizome extract was also active (1 cm/g) against two Penicillium species. This activity is not lost upon lyophilization of extracts, or extended storage (6 months) at room temperature or -20°C, or boiling upon suspension in 10 mM Tris buffer pH 8. After fractionation of the active principle on TLC with three solvent systems, UV-Vis spectrophotometric analyses of the methanol eluant from silica TLC plates show a single peak at 277 nanometers (nm).

Sprout tissue and basic extracts from Coffea show some activity (>1 cm/g fresh sprout tissue for basic extract). Cobioautography of extracts from coffee sprouts with pure caffeine demonstrates an activity against P. camemberti that migrates differently than caffeine. This activity was lost upon storage of the basic extract at -20°C.

| TABLE 1. Variable resistance of nonsterilized sprouts to molding when placed on microbial medium. |
|--------------------------------------------------|------------------|
| Resisted molding                                | Did not resist molding |
| Amaranthus/ Sprout                              | Asparagus         |
| Chervil/ Sprout                                 | -                 |
| Coffee/ Sprout                                  | +/−               |
| Lotus/ Sprout                                   | A. niger, +       |
| Lotus/ Rhizome                                  | A. niger, ++      |

Notes:
- E. coli, S. epidermidis.
- Antifungal activity: - no activity, +/- slight activity, + activity, ++ strong activity.
DISCUSSION

Many screening efforts for novel antimicrobial agents have focused on plants used in folk medicine (11). Most of these plants are not edible. Because of our interest in food preservation we have screened edible plants, using plants or plant parts which may have a greater ecologic need for antimicrobial defense. We report at least seven activities from 18 plant sprouts and 1 plant rhizome by using combinations of common methods. Bias in material selection and wide variation in the efficacies of screening methods employed by researchers (12) preclude direct comparison of this approach to the success of other strategies. Most of the activities from sprouts are weak and of narrow antimicrobial spectrum, and thus appear to have little or no promise for food preservation. Contrarily, the activity in the edible parts of lotus and coffee merits investigation.

Bioautography demonstrates an antifungal from coffee sprouts distinct from free caffeine. Demonstration of the active principle's nonidentity to bound caffeine or other purine alkaloids (such as theobromine or theocrine) has not been attempted. The loss of this activity upon storage at -20°C has stopped us from redemonstration and further investigation of this effect.

The activity from the rhizome of lotus is relatively potent. This activity was extracted from commercially available rhizomes of unknown age or origin or physiologic state. Rhizomes, obtained approximately one year apart, show the same activity. This antifungal factor inhibited the growth of all filamentous fungi used in this study. The activity is stable for long periods of time at room temperature and resistant to boiling. Spectrophotometric analyses suggest we have isolated a single compound, but this absorption maximum of 277 nm does not allow speculation on the chemical nature of this activity. We suggest other spectrophotometric methods will aid elucidation of the chemical nature of the active principle in lotus. Screening of related aquatic species for antimicrobial activities may be a fruitful approach to finding other antimicrobials.

The Holy Lotus, *Nelumbo nucifera*, is widely eaten in the Far East, particularly in China, Korea, Indonesia, and Thailand. The seeds are eaten fresh and candied, and the rhizome is consumed as a fresh vegetable as well as prepared and packaged in a variety of ways. The rhizome is exported in large quantity. A related water lily, *Nelumbo lutea*, the American yellow-flowered lotus is also edible but has not been investigated for antifungal activity.

We have studied a select few edible plants, pursuing either leads reported by others or exploring some niches which looked promising. There are many other edible plants which could be explored for antimicrobial constituents.

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