Journal of Food Protection, Vol. 49, No. 5, Pages 340-341 (May 1986)

A Research Note

Antimicrobial Activity of (+)-Tuberine

SAID O. GNAN* and G. M. SHERIHA

University of Al-Fateh and Medicinal Plant Research Program, NASR, Tripoli, Lybia

(Received for publication August 14, 1985)

ABSTRACT

A new alkaloid, (+)-tuberine isolated from *Haplophyllum tuberculatum,* had high antimicrobial activity against *Staphylococcus aureus, Bacillus subtilis* and *Saccharomyces cerevisiae* at 1 μg/ml. (+)-Tuberine was slightly inhibitory to *Escherichia coli.*

As part of an on-going investigation of phytochemical agents in folk medicinal plants of Libyan flora, Sheriha and Abouamer (*4*) isolated and structurally characterized several 1-aryl-2,3-naphtalide lignans. One fraction extracted from *Haplophyllum tuberculatum (Rutaceae)* was an alkaloid (Fig. 1) they named (+)-tuberine. Sixty-nine African *Strychnos* species have been screened for the presence of tertiary alkaloids (*1*). *Strychnos afzelii* Gilg inhibited streptococci (*6*). Among the *Rutaceae* family of plants, antitumor and antimicrobial activity were reported for *Aletes acualis, Angelica brevicaulis, Anthriscus negalecta* and *Lenium maculatum (6).* Oregano, rosemary, sage and thyme in concentrations of 0.5 to 8 g/L delayed growth and acid production by *Lactobacillus plantarum* and *Pediococcus acidilactici* in broth (*7*). Limonene suppressed growth and oxygen uptake by *Staphylococcus aureus (2),* and essential oils of orange and lemon were more inhibitory to yeast than to bacteria (*5*).

MATERIALS AND METHODS

We obtained *Saccharomyces cerevisiae, Bacillus subtilis* and *Escherichia coli* from the University of Al-Fateh-Tripoli, Libya, and *Staphylococcus aureus* from Washington State University (*3*). These organisms were selected as representing a variety of foodborne microorganisms. They were exposed to (+)-tuberine at concentrations of 0.1 and 1.0 μg/ml after preliminary experiments revealed the microorganisms were completely inhibited by 48 μg/ml. Stock cultures of bacteria and yeast were prepared weekly in nutrient broth and potato dextrose broth, respectively.

Tests of sensitivity to (+)-tuberine were done using disk assays and exposure in broth with measurements of response by plate counts and turbidity measurements.

For disk assays, petri dishes (10 cm diameter) were poured with 20 ml of nutrient agar (pH 7.0) for bacteria or potato dextrose agar (pH 4.5) for yeast, and inoculated with sufficient freshly prepared cells (10^7 to 10^8 CFU/ml) to yield a lawn of growth. After solidification, filter disks (1 cm diameter) were dipped in methanol (negative control), (+)-tuberine (0.1 and 1.0 μg/ml) or erythromycin (2 μg/ml) and placed in separate quadrants of each of three plates. After incubation at 37°C for 24 to 48 h, zone sizes were measured under 10× magnification. Zone sizes reported were the total diameter minus the diameter of the small zone produced by methanol.

For plate count cultures, 10^7 to 10^8 CFU/ml were added to sterile nutrient broth (bacteria) or potato dextrose broth (yeast) containing 0, 0.1 and 1.0 μg (+)-tuberine/ml, and incubated at 37°C for 24 h using an incubator shaker.

For turbidity measurements, nutrient or potato dextrose broths in Erlenmeyer flasks (100 ml/250 ml flask) were prepared with 0, 0.1 and 1.0 μg of (+)-tuberine/ml and sterilized at 121°C for 15 min. An 18- to 24-h culture (0.5 ml) of the test cultures (10^7 to 10^8 CFU/ml) was added to each cooled flask. After incubation at 37°C for 12 h on a shaker, absorbance was measured at 520 nm with a Bausch and Lomb Spectronic 20 spectrophotometer. Percentage inhibition was calculated using the following formula:

$$\% \text{ inhibition} = \frac{(\text{control} - \text{control blank}) - (\text{treatment} - \text{treatment blank})}{(\text{control} - \text{control blank})} \times 100$$

RESULTS AND DISCUSSION

The 1.0 μg/ml concentration of (+)-tuberine was highly inhibitory to both *S. aureus* and *B. subtilis,* moderately inhibitory to *S. cerevisiae,* and slightly inhibitory to *E. coli* (Table 1).

In plate count studies, the initial numbers of 0.3 to 0.5 × 10^6 CFU of bacteria/ml increased in control broth to numbers too numerous to count at the 10^{-6} dilution when incubated at 37°C for 24 h. However, in broth with 1.0 μg (+)-tuberine/ml, numbers of *B. subtilis* and *S.*

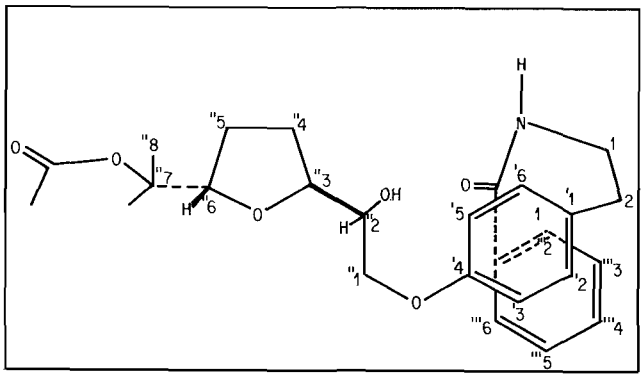

Figure 1. *Structure of n-benzoyl-4'-[(2''S, 3'' S, 6'' S) - (+) - 7'' - acetoxy - 2'' - hydroxy-3'', 7'' - dimethyl - 3'', 6'' - epoxy-octyloxylphenethyl-amine [(+)-tuberine].*

TABLE 1. *Relative inhibitory activities of (+)-tuberine and erythromycin in disk zone inhibition tests (n=4).*

	Treatment		
	(+)-tuberine		Erythromycin
Microorganism	0.1 μg/ml	1.0 μg/ml	2 μg/ml
S. aureus	+[a]	+++	+
E coli	−	+	+
B. subtilis	+	+++	+++
S. cerevisiae	+	++	−

[a]Zone sizes: −, none; +, 2 to 5 mm; ++, 6 to 10 mm; +++, 10 to 15 mm.

aureus increased slowly for 8 h, then became too few to count at the 10^{-6} dilution by the end of 24 h at 37°C (Table 2). *S. cerevisiae* as well was inhibited by 1 μg (+)-tuberine/ml and *E. coli* was inhibited marginally.

In turbidity measurement studies, each test organism was inhibited by (+)-tuberine, but *E. coli* to a lesser extent than the other three (Table 3).

Hence, results of the three methods of assay were in agreement, showing that (+)-tuberine is relatively effective as an inhibitor of *B. subtilis, S. aureus* and *S. cerevisiae*. Inhibition by 1.0 μg of (+)-tuberine/ml is especially noteworthy in view of the relatively high concentrations of orange oil (2 mg/ml) and herbs (0.5 to 8 mg/ml) previously reported to be somewhat inhibitory to microorganisms.

TABLE 2. *Plate counts of selected microorganisms incubated in broth containing (+)-tuberine (n=3).*

		CFU/ml × 10^{-6}		
Micro-organism	Time intervals (h)	0.1 μg/ml	1 μg/ml	Control
B. subtilis	0	0.3	0.2	0.4
	4	13	9	30
	8	300	70	800
	24	300	-[a]	TN[b]
E. coli	0	0.2	0.1	0.3
	4	20	10	30
	8	300	20	TN
	24	TN	2000	TN
S. aureus	0	0.3	0.3	0.5
	4	20	10	30
	8	800	400	3000
	24	2000	-	TN
S. cerevisiae	0	0.07	0.06	0.08
	4	1	-	5
	8	6	-	300
	24	-	-	

[a]-, $<10^6$/ml.
[b]TN, too numerous to count.

TABLE 3. *Antimicrobial activity of (+)-tuberine tested by turbidity measurement (n=3).*

	% Inhibition	
Microorganism	0.1 μg/ml	1 μg/ml
B. subtilis	84	86
E. coli	48	50
S. aureus	85	94
S. cerevisiae	84	91

REFERENCES

1. Bisset, N. G., and J. D. Phillipson. 1971. The African species of *Strychnos*. Part II. Alkaloids. Lloydia 34:1-60.
2. Gnan, S. O., and S. M. Rana. 1983. Antimicrobial activity of limonene. Libyan J. Agr. 11:99-102.
3. Hunt, G. A., A. Gourvilch, and J. Lein. 1958. Preservation of cultures by drying on porcelain beads. J. Bacteriol. 76:453-454.
4. Sheriha, G. M., and K. M. Abouamer. 1984. Lignens of *Haplophyllum tuberculatum*. Phytochemistry 23:151-153.
5. Subba, M. S., T. C. Soumithri, and R. Susyanarayana Rao. 1967. Antimicrobial action of citrus oils. J. Food Sci. 32:225-227.
6. Vepoorte, R., E. W. Kde, H. Van Doorne, and A. Baerheim Svendsen. 1978. Antimicrobial effect of alkaloids from *Strychnos afzelii* Gilg. Planta Medica 33:237-242.
7. Zaika, L. L., J. C. Kissinger, and A. E. Wasserman. 1983. Inhibition of lactic acid bacteria by herbs. J. Food Sci. 48:1455-1459.