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Antibacterial Activity of Isolates from *Piper longum* and *Taxus baccata*

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Abstract

This study describes the antibacterial activity of the pure isolates from *Piper longum* (L.) (black pepper) and *Taxus baccata* (L.) (Yew). Three isolates of black pepper were active against Gram-positive bacteria and moderately active against Gram-negative bacteria. Each isolate was highly active against at least one particular species of bacteria; piperlonguminine (**1**) against *Bacillus subtilis*, piperine (**2**) against *Staphylococcus aureus*, and pellitorine (**3**) against *Bacillus sphaericus*. 3-(3′-4′-5′-Trimethoxyphenyl) propionicacid (**4**) did not show any antibacterial activity. The isolate (–)-rhododendrol (**5**) of *Taxus baccata* (L.) inhibited *Salmonella typhimurium* and *Pseudomonas syringae*, while 4-(4′-hydroxyphenyl)-butan-2-one (**6**) and 4-(4′-hydroxyphenyl)-trans-but-3-en-2-one (**7**) inhibited *Pseudomonas syringae* and *Bacillus sphaericus*. It is therefore evident that all the isolates of *Piper longum* had antibacterial activity except 3-(3′-4′-5′-trimethoxyphenyl) propionicacid (**4**), whereas isolates of *T. baccata* showed moderate activity.

Keywords: Anti-bacterial activity, *Bacillus sphaericus*, *B. subtilis*, *Escherichia coli*, minimum inhibitory concentration, *Pseudomonas syringae*, *Piper longum* (L.), *Salmonella typhimurium*, *Staphylococcus aureus*, *Taxus baccata* (L.).

Introduction

Plants exhibit a natural resistance to bacterial diseases. Such resistance depends on many mechanisms that act at different levels of infection, among which, the synthesis of antimicrobial substances is particularly important. The antimicrobial substances isolated so far from plants are active at high concentrations (Barnabas & Nagarajan, 1988).

Piper longum (*L.*) (Piperaceae) and plants belonging to the genus *Piper* are common in the Indian Ayurveda system of medicine (Krishnamurthy, 1969). It has long been used as medicine in rural and tribal areas in the world. The dried fruit and root in the form of a decoction is extensively used in rural areas for acute and chronic bronchitis accompanied by coughing. The ripe fruit is sweet, pungent, laxative, antidiarrhoeic and antidysenteric. It is also widely used as popular medicine to cure diseases like leprosy and tuberculosis (Kirtikar et al., 1950). Aduncamide, a new amide isolated from *Piper aduncum* showed anti-microbial activity against *Bacillus subtilis* and *Micrococcus leteus* (Orijala et al., 1993). Eugenol isolated from *Piper betle* exhibited strong anti-microbial activity (Nair & Bruke, 1990). Propenyl phenols from *Piper sarmentosum* were reported to have inhibitory activity against *Escherichia coli* and *Bacillus subtilis* (Masuda et al., 1991).

Taxus baccata (*L.*) belongs to the Taxaceae. Leaves of *Taxus* are used for nervousness, hysteria, epilepsy and as a lithontriptic. A tincture made from young shoots has long been in use for headache, giddiness, feeble, and falling pulse and diarrhea. An aqueous extract of leaves showed a depressant effect on the central nervous system of rats indicating the presence of transquillizing principle (Ambasta, 1994). Yew extracts have hormonal activity in insects and mammals.

The chemistry of the yew plant has gained significant interest in recent years because of taxane diterpenoids and some non-alkaloid taxoids (Gu et al., 1991). Among the phenolic constituents of yew, the butanoid glucoside rhododendrin also shows hepatoprotective activity (Parmar et al., 1991).

In the present investigation, we have studied the potential effects of plant extracts as antibacterial agents.

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Materials and Methods

Plant material

The fruits of *Piper longum* (L.) (500 g) were collected from Andhra Pradesh, India in August 1995. The needles of *Taxus baccata* (L.) were collected from Jammu in May 1997. The plants were identified by the herbarium of the natural products laboratory, IICT, Hyderabad, India. Voucher specimens (PL-F) and (TB-NJ) were preserved, respectively.

Extraction and isolation

The dried fruits (500 g) of *Piper longum* (L.) were powdered and extracted with CH_2Cl_2-MeOH (1 : 1) for 120 hr. The extract was concentrated (150 g) under reduced pressure and chromatographed over silica gel (30 g), the column being eluted with petroleum ether and petroleum ether-ethyl acetate (EtOAc) mixtures. The following compounds were eluted according to the increasing polarity: pellitorine (**3**) (147 mg), piperlonguminine (**1**) (110 mg), piperine (**2**) (138 mg) and 3-(3′-4′-5′-trimethoxyphenyl) propionicacid (**4**) (408 mg) (Das et al., 1998a).

Air-dried needles of *Taxus baccata* (1 kg) were powdered and extracted three-times with CH_2Cl_2-MeOH (1 : 1). Each extraction was continued for 72 hr. The combined extract was filtered and the solvent removed from the filtrate under reduced pressure to yield a dark green residue (32 g). The extract was chromatographed over silica gel (30 g) and the column was eluted with solvents of increasing polarity-using hexane-EtOAc in different proportions as solvent system. The following compounds were eluted according to the increasing order of polarity: (−)-rhododendrol (**5**) (256 mg), 4-(4′-hydroxyphenyl)-butan-2-one (**6**) (43 mg) and 4-(4′-hydroxyphenyl)-trans-but-3-en-2-one (**7**) (32 mg) (Das et al., 1998b). The structure and identification of purified compounds were performed by analysis of spectroscopic data (UV, IR, ^{1}H-NMR and GCMS) and compared with those previously described in the literature.

Antibacterial assay

Bacterial strains obtained from the Institute of Microbial Technology (Chandigarh, India) were employed in the assay. They included the Gram-positive bacteria *Bacillus sphaericus* (ATCC # 14577), *B. subtilis* (ATCC # 6051), *Staphylococcus aureus* (ATCC # 9144), and the Gram-negative bacteria *Escherichia coli* (ATCC # 25922), *Pseudomonas syringae* (ATCC # 13457) and *Salmonella typhimurium* (ATCC # 23564). Bacterial strains were grown on Mueller-Hinton (MH) agar plates (Hi-Media, India) and suspended in MH broth. The minimum inhibitory concentration (MIC) values against bacterial strains were obtained using a broth macro-dilution method (Ericsson & Sherris, 1971). Antibacterial test solution was prepared by dissolving the compound in dimethylsulphoxide (DMSO) and diluted in Muller-Hinton broth to highest final concentration desired. The inoculum suspensions were prepared from 6 hr broth cultures and adjusted so as to obtain turbidity equivalent to 0.5 McFarland turbidity standard. Serial ten-fold dilutions were made to obtain a concentration range of 0.01–200 µg/ml for the plant extracts. Two-fold dilutions of test compounds in the range of the minimal concentrations showing activity were tested to obtain a more precise measure of the MIC. The bacterial suspensions were aerobically incubated for 24 hr at

Table 1. Antibacterial activity of chemical isolates from *Piper longum* and *Taxus baccata*[a].

	Compounds tested							
Microorganisms	**1**	**2**	**3**	**4**	**5**	**6**	**7**	PEN-G
Gram-positive								
Bacillus sphaericus	20	25	25	>200	160	110	100	34
Bacillus subtilis	9	12	12.5	>200	140	>200	190	32
Staphylococcus aureus	12.5	12.5	20	>200	150	>200	>200	0.05
Gram-negative								
Escherichia coli	150	160	150	>200	150	>200	190	64
Pseudomonas syringae	75	50	75	>200	90	110	110	**NA**
Salmonella typhimurium	175	180	200	>200	80	>200	>200	4

[a] MIC values (µg/ml) determined from the mean of three experiments.
NA = absence of inhibition even at the highest concentration used.
1 = piperlonguminine; **2** = piperine; **3** = pellitorine;
4 = 3-(3′-4′-5′-trimethoxyphenyl)-propionic acid;
5 = (−)-rhododendrol; **6** = 4-(4′-hydroxyphenyl)-butan-2-one;
7 = 4-(4′-hydroxyphenyl)-*trans*-but-3-en-2-one.
PEN-G = benzyl penicillin sodium.

37 °C. The MIC was defined as the lowest concentration able to inhibit any visible bacterial growth. Sterile DMSO, which did not influence bacterial growth, was used as control. The MIC values were also determined for benzyl penicillin sodium in MH broth as a positive control.

Results and Discussion

The MICs of *Piper longum* are presented in Table 1. Piperlonguminine (**1**) showed the highest anti-bacterial activity against *B. subtilis* at 9 µg/ml. All the compounds except 3-(3′-4′-5′-trimethoxyphenyl) propionicacid (**4**) showed potent activity against *P. syringae* and moderate activity against *E. coli* and *S. typhimurium*.

The MICs of the three compounds from *T. baccata* are presented in Table 1. (–)-Rhododendrol (**5**) showed highest inhibition when tested against *P. syringae* at 90 µg/ml. *S. typhimurium* was inhibited at a concentration of 80 µg/ml for (–)-rhododendrol (**5**). *Bacillus sphaericus* was inhibited by 4-(4′-hydroxyphenyl)-butan-2-one (**6**) at 110 µg/ml, while 4-(4′-hydroxyphenyl)-*trans*-but-3-en-2-one (**7**) inhibited at 100 µg/ml; these agents were more active than (–)-rhododendrol (**5**) which had an MIC value of 160 µg/ml.

It was found that the compounds of *P. longum* and *T. baccata* showed potent inhibition against Gram-positive bacteria. In *Bacillus subtilis*, the MIC was 9 µg/ml for piperlonguminine (**1**), 12 µg/ml for piperine (**2**), and 12.5 µg/ml for pellitorine (**3**), as compared to 32 µg/ml for penicillin G which was highly significant. Similarly, the compound tested against *S. aureus* and *B. sphaericus* also showed inhibition, but activity was as intense as benzyl penicillin sodium. Three compounds showed effective inhibition when tested against the Gram-negative bacterium *P. syringae*, but was ineffective against *E. coli* and *S. typhimurium*; earlier workers (Yamaguchi & Ozeki, 1985) found only piperine (**2**) active against *P. aeruginosa* and *Alcaligenes* F 2518.

These results warrant further research into the role of the compounds as antibacterial agents, their respective binding sites and mode of action.

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