Effect of Plant Flavonoids on Bacteria

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Abstract: The ethyl acetate soluble fractions of methanol extracts of six mangrove plants and seven isolated flavonoid compounds from them were tested for antimicrobial activity using the agar diffusion method against ten types of bacteria. The ethyl acetate extracts of all the species showed activity against *Bacillus subtilis and staphylococcus aureus. Bruguiera parviflora* leaf extract showed the highest activity against *B. subtilis* and S. *aures* with an inhibition zone of 15.6 mm and 18.9 mm at concentration 50 mg/mL. Of the isolated compounds tested, 4′, 5′, 7- trihydroxy- 3′, 5- dimethoxy flavone was found to be effective against the higher types of bacteria (seven types) with an average inhibition zone 12.0 mm at concentration 2.5 mg/mL.

Key words: Flavonoid, agar diffusion method, Bruguiera parviflora, 4′, 5′, 7- trihydroxy- 3′, 5- dimethoxy flavone.

Introduction

Plants produce phytoalexins which are antimicrobial metabolites. Many of these phytoalexins are flavonoids [1]. It was examined the effects of 24 phenolics, including anthocyanins, leucoanthocyanins and phenolic acids, on ten types of becateria and found that most of the compounds inhibited their respiration and reproduction. More than twenty flavonoids have been found show various degrees of antibacterial activity [2,3].

From chemical studies, it was found that large amounts of phenolic compounds are present in mangrove plants. One of the major biological properties of phenolic compounds is their antimicrobial activity and their main role in plants is to act as protective compounds against disease agents such as fungi, bacteria and viruses [4,5,6,7,8].

For this, mangrove plants are of special interest in the search of biologically active compounds. Since 1950, the chemical constituents of some common genera were investigated, but a few biological activity studies of mangrove plants have been reported [4],

The present study was carried out for the first time to determine the antimicrobial activity on bacteria of the extracts of six mangrove plants and of some isolated flavonoid compounds from them.

Materials and methods

Plant material

Laves of mangrove plants of Brugiera gymnorrhiza (L) Lam. (Rhizophoraceae), Bruguiera cylindrica (L) B1. (Rhizophoraceae), Bruguieara parviflora (Roxb.) W and A. ex Griff. (Rhizophoraceae), *Avicennia marina* (Forsk.) Vier. var. resinifera (Forst.) Back. (Avicenniaceae), *Avicennia officinalis* L. (Avicenniaceae) and *Avicennia alba* Blume.

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(Avicenniaceae) were collected from the mangrove swamp of Batu Maung, Pulau Pinang, Peninsular Malaysia. The plants were indentified by the School of Biological Science, Universiti Sains Malaysia, Malaysia.

Extraction

Leaves were dried in an oven at 40°C and crushed into powder. The powdered leaves were first extracted with petroleum ether and then with methanol. The methanol extracts were evaporated and separated into chloroform, ethyl acetate and butanol soluble fractions. The ethyl acetate soluble fractions was evaporated and subjected to bioassay. Again, seven compounds - 4′, 5′, 7- trihydroxy - 3′, 5- dimethoxy flavone, chrysoeriol, isovitexin, quercetin, rutin, biochanin A-7-0- xylosyl glucoside and luteolin, were isolated and identified from the ethyl acetate extract of plants [2], and tested for antimicrobial activity. Test solutions of different concentrations were made up in phosphate buffer (p^H = 8.0).

Antimicrobial Screening

The agar diffusion method was used for the bioassay. The following microorganisms (laboratory strains) were obtained from the School of Pharmaceutical Sciences, Universiti Sains Malaysia, Malaysia and used for this test: *Bacillus subtilis, Staphylococcus. aureus, Bacilhis mirabilis, Streptococci, Salmonella typhi, Salmonella paratyphi, Enteropbacter aerogenase, Proteus vulgaris, Bacillus globigii and Proteus rettgeri.*

The microorganisms were subcultured from the stock culture overnight at 37°C in sterilized nutrient broth (Oxoid). Each culture was suitably diluted with sterilized nutrient broth to give a suspension of about 10^8 micro- organism per ml. For each plate, 0.1 mL of broth culture of test organism was pipetted into 25 mL of sterilized molten antibiotic agar medium No. 1 (Merch) in bottle at 45°C and the mixture was poured into sterilized petri dishes (dia. 9.0 cm) and allowed to set at room temperature. In each plate, 4 holes with a diameter of 7 mm each were made by using a sterile cork borer. Test solution (0.15 mL) was dispensed into hole. The plates were left at room temperature for 3 hours after which they were incubated at 37°C for 18 hours. The zone of inhibition was measured with a vernier caliper.The bioassay consists of two parts. Firstly, ethyl acetate extract of six species and seven isolated compounds were tested separately on ten types of bacteria. Secondly, comparison of antibacterial activity of ethyl acetate extracts of six species and two standard antibiotics, neomycin and gentamycin (Sigma) were carried out. The first experiment was performed in duplicate and later for comparison, in six replicates. In both experiments, the controls showed no zone of inhibition. The results are shown in Tables 1, 2, 3 and 4.

Results and discussion

Evaluation of the antimicrobial activities on ten types of bacteria were carried out separately with the ethyl acetate soluble fraction of the methanol extract of the leaves of six mangrove plant species at various concentrations (50, 25, 10, 5 mg/mL). Seven

isolated flavonoid compounds at various concentrations (2.5, 1.0.5, 0.25 mg/mL) were similarly assayed on ten types of bacteria and the results of both types of experiments are shown in Tables 1 and 2.

Table 1: *Antimicrobial activity of the ethyl acetate extracts of six mangrove plants.*

Bacteria	Plant Species					
	I	II	III	IV	V	VI
B. subtilis	+++	++	++	++	++	++
S. aureus	++	++	++	++	++	+++
Pr. mirabilis	++	±	++	±	++	-
Streptococci	±	±	±	±	±	±
S. typhi	±	-	±	-	-	±
S. paratyphi	++	-	±	-	-	-
Ent. aerogenase	±	-	-	-	-	-
Pr vulgaris	++	++	-	±	++	±
B. globigii	++	++	-	±	±	-
Pr. rettgeri	-	+	++	-	-	-

1 : B. gymnorrhiza, 11 : B. cylindrica, 111 : B. parviflora, IV : A. marina, V : A. officinalis, VI : A. alba.

The activity was tested by means of the agar diffusion method (see experiment) at various concentrations (0 : 15mL containing 50, 25, 10 and 5 mg plant extract per mL).
Grading of the results;

- \- no activity.
- ± activity at 50mg/ mL, inhibition zone < 10mm.
- \+ activity at 50mg / mL, inhibition zone > 10mm.
- ++ activity at 50mg / mL and 25mg/ mL, inhibition zone at 25mg/mL > 8mm.
- +++ activity at 50mg / mL, 25mg/ mL and 10mg/mL, inhibition zone at 10mg/ mL > 7.5mm.

Table 2: *Antimicrobial activity of the isolated compounds.*

Bacteria	Plant Species						
	I	II	III	IV	V	VI	VII
B. subtilis	++	±	++	++	++	+++	++
S. sureus	++	++	++	++	++	+++	++
Pr. mirabilis	++	-	-	+++	±	++	++
Streptococci	+	-	±	++	±	±	±
S. typhi	-	-	±	±	++	-	-
S. paratyphi	-	-	-	-	±		-
Ent. aerogenase	++	-	-	-	-	±	-
Pr. vulgaris	++	±	++	-	++	++	++
B. globigii	+++	±	++	-	±	±	+
Pr. rettgeri	-	-	-	±	±	-	-

1 : 4′, 5′, 7- trihydroxy - 3′ 5- dimethoxy flavone, 11 : chrysoeriol, 111 : isovitexin, IV : quercetin, V : rutin, VI : biochania A-7-0- xylosyl glucoside, VII : luteolin.

The activity was tested by means of agar diffusion method at various concentrations (0.15mL containing 2.5, 1.0 0.5 and 0.25 mg compound per mL).
Grading of the results:

- no activity
± activity at2.5mg/mL, inhibition zone < 10mm.
+ activity at 2.5mg /mL, inhibition zone > 10mm.
++ activity at 2.5mg /mL and 1 .Omg/ mL, inhibition zone at 1.0mg/mL> 8mm.
+++ activity at 2.5mg /mL, 1.0mg/ mL and 0.5mg/mL, inhibition zone at 0.5mg/ mL >7.5mm

The tests show that ethyl acetate extracts of all the species are active. Of the solated compounds tested, 4', 5', 7- trihydroxy -3', 5- dimethoxy flavone was the most active against the higher types of bacteria (seven types).

Later, *B. subtilis* and S. *aureus* were selected for use in evaluation of the relative antimicrobial activity of the ethyl acetate extracts. This was because the ethyl acetate extracts of all the species studied showed activity against these two types of bacteria. 8. *parviflora* leaf extract showed the highest activity against both bacteria types, with an inhibition zone of 15.60 mm 18.90 mm at conc. 50 mg/mL. On the other hand, gentamycin showed a higher activity with an inhibition zone of 16.87 mm and 17.83 m at conc. 50 pg/mL (Tables 3 and 4).

Table 3: *Antimicrobial activity produced by the ethyl acetate extract of different species and standard antibiotics on* Bacillus subtilis *[Mean + S.E.M. diameter of zone inhibition (mm)].*

Plant species	100 mg/mL	50 mg/mL	25 mg/mL	10 mg/mL
B. gynorrhiza	14.04 ± 0.21	11.48 ± 0.18	09.40 ± 0.13	08.07 ± 0.11
B. cylindrica	10.08 ± 0.07	09.57 ± 0.09	08.23 ± 0.26	NA
B. parviflora	17-77 ± 0.14	15.57 ± 0.19	12.59 ± 0.21	10.15 ± 0.12
A. marina	13.07 ± 0.11	10.90 ± 0.20	08.32 ± 0.07	NA
A. officinalis	16.67 ± 0.16	13.97 ± 0.28	10.28 ± 0.70	NA
A. alba	17.17 ± 0.17	13.62 ± 0.26	11.25 ±0.24	09.25 ± 0.07

Antibiotics	HD (50 pg/mL)	LD (10 ug/mL)
Neomycin	14.32 ± 0.12	10.52 ± 0.30
Gentamycin	16.87 ± 0.20	12.82 + 0.20

Table 4 : *Antimicrobial activity produced by the ethyl acetate extract of different species and standard antibiotics on Staphylococcus aureus [Mean + S.E.M. diameter of zone inhibition (mm)].*

Plant species	100 mg/mL	50 mg/mL	25 mg/mL	10 mg/mL
B. gynorrhiza	17.43 ± 0.31	16.00 ± 1.02	13.80 ±0.18	09.10 ± 0.01
B. cylindrica	14.33 ± 0.19	12.27 ± 0.15	10.45 ± 0.14	NA
B. parviflora	20.40 ±0.18	18.95 ± 0.11	16.20 ± 0.15	12.52 ± 0.14
A. marina	14.93 ± 0.16	11.92 ± 0.14	09.28 ± 0.09	NA
A. officinalis	13.18 ± 0.11	10.18 ± 0.08	08.63 ± 0.09	NA
A. alba	15.77 ± 0.12	14.38 ±0.15	12.15 ±0.09	08.98 ± 0.07

Antibiotics	HD (50 ug/mL)	LD (10 pg/mL)
Neomycin Gentamycin	15.77 + 0.10 17.83 + 0.22	12.01 +0.11 14.85 + 0.13

Therefore, the antibiotic is more or less 1000 times more effective than the ethyl acetate extract of *B. parviflora* leaves. Hedin and Wages studied antibacterial activity of 63 known flavonoids with *P. maltophila* and *E. cloacae* [10]. Most of the compounds showed activity of about 7.0 mm at conc. 50 mg/mL. In comparison to our work, although the test organisms are different, the isolated compound, 4′, 5′, 7- trihydroxy - 3′, 5- dimethoxy flavone, appears to be 50 times more active (9.0 mm at conc. 1.0 mg/mL).

Although, the activities of crude extracts of mangrove plants and their isolated flavonoids are far from striking, our studies suggest that there may be some potent antimicrobial flavonoids from plants after slight modifications to molecular structure or substituents.

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References

1. Perrin D.R and BottomLey W., 1968, *J. Amer Chem.* Soc. 84, PP 1919-1922.
2. Powers. J.J., 1964, Fourth International Symposium on Food Microbiology, Sweden, PP. 54-75.
3. Laliberte R., Campbcll D. and Bruderlein F., 1967, *Can. J Pharma. Sci.*, 2, 37.
4. Weber D. J. and Hess W. M., 1976, The Fungal Spore: Form and Function, New York, PP 73-98.
5. Harbone J. B, Ingham J. L and King L., 1976, *Phytochemistry*, 15, PP 1485-1487.
6. Preson N. W., 1977, *Phytochemistry*, 16, PP 143-144.
7. Pinkas J., Lavie D. and Chorin M., 1968, *Phytochemistry*, 7, PP. 169-174.
8. Johnson G. and School L. A., 1952, *Science*, 115, PP. 627-629.
9. Abu Raihan S. M., 1989, *Studies on the Chemical constituents and Biological Activities of Some Mangrove Plants.* Ph.D. thesis, Universiti Sains Malaysia, Malaysia.
10. Hedin P. A. and Waage S. K., 1987, Proceedings of the UNESCO Regional Seminar on the Chemistry of Mangrove Plants, Bangkok, Thailand, May 14-16, p 68.