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AN EVALUATION OF THE ANTIFUNGAL ACTIVITY OF THE CRUDE EXTRACT OF THE HEMICHORDATE *PTYCHODERA FLAVA* OF THE MADRAS COAST, INDIA*

HILDA AZARIAH, S. RAJAN** AND JAYAPAUL AZARIAH**

*Centre for Advanced study in Botany and **Department of Zoology, University of Madras, Guindy campus, Madras 600 025*

ABSTRACT

It is known that the extracts of various marine organisms exhibit antibacterial and antifungal activities. A number of studies has been carried out on the ecophysiology of the hemichordate *Ptychodera flava* of the Madras Coast. Such studies have brought to light the presence of bio-active compounds in the crude extract of the hemichordate. It has been reported that the extract, in high concentrations, inhibited the clevage of the fertilized cell of the marine polychaete *Hydroides elegans* whereas low concentrations of the extract accelerated the clevage process.

The present study was undertaken to test the antifungal activity, if any, in the crude extract of *P. flava*. The fungus *Pestalotia palmarum*, a leaf blight pathogen in coconut palm and tea plant was choosen as the bioassay material. Using axenic cultures, the effect of the crude extract of *P. flava* on the fungal spore germination and mycelial growth have been investigated. Pharmacological significance of the investigation has been commented upon.

INTRODUCTION

IN RECENT years much interest has been evinced in searching the sea for bioactive compounds and in studying their mode of action on various pathogenic microbes. It has been reported that the various marine organisms exhibit antibacterial and antifungal activity. Humn and Lane (1974) have summarised the antimicrobial and lethal properties of the aqueous extract of *Haliclona viridis* (sponge). The active factor has been named as 'Halitoxin'. Sharma and Burkholder (1967) isolated antibacterial substances from *Verongia cauliformis* (sponge) which was active against a wide spectrum of bacteria. It is known that the crude extract of *Antillorgorgia turgida* (Coelenterata) has an antibacterial property. Similarly, the extract of the polychaete worm *Thelephus setosus* exhibited an active factor — Thelpin which is effective as an antifungal agent. The active factor in the extract of *Stychopus japanicus* (sea-cucumber), termed as 'Holotoxin', has an antifungal activity (Shimada, 1969).

The crude extract of *Ptychodera flava* has been known to contain bioactive compounds, which exhibited bioactivity against the growth of diatoms and the fertilized eggs of polychaetes (Azariah *et al.*, 1978 and Azariah and Pillai, 1985). An exploratory attempt has been made to assess the presence of antifungal activity, if any, of the extract by using pure cultures of the fungus *Pestalotia palmarum*, a leaf blight pathogen in coconut plants and tea plants.

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MATERIALS AND METHODS

The fungus *P. palmarum* isolated from leaves of coconut, was used as a bioassay organism. The fungus was grown on potato dextrose agar (PDA) medium (Ricker and Ricker, 1936) at room temperature. Sporulation of the fungus was achieved by exposing the colony to near UV radiation from two BLB lamps (Swamy and Mani, 1978).

The aqueous conidial suspension from 8-10 day old cultures were obtained by sterile filtration through cheese cloth, washed three times with sterile glass distilled water at 5000 rpm for 15 minutes to wash away the self inhibitors. The concentration of the conidia in suspension was adjusted to 2×10^6 conidia / ml using a Thoma Haemocytometer and then they were used for the germination assay.

Preparation of the extract of P. Flava

The extract of *P. flava* was prepared by the method of Azariah *et al.* (1978). An animal was suspended in 100 ml of distilled water and was considered as the 100% concentration of the extract. Further, working solutions were prepared by diluting the mother extract with distilled water.

Spore germination technique

A drop of spore suspension of the test fungus (2×10^6 conidia/ml) was added to 1 ml of various concentrations of the extract (200, 100, 50, 25, 12, 6, 3, 1, 0.5, 0.1, 0.01, 0.001 and 0.0001 %) and mixed well. Three drops of the spore suspension, mixed with the extract, were placed on a cavity slide at room temperature. Spore suspension in distilled water was kept as control. After 12 hr incubation in a moist chamber, the slides were examined for the spore germination.

Mycelial growth

The extract of *P. flava* was added to double concentration of PDA medium so as to arrive at a final concentration of 100%, 50% and 25% strength of the extract in PDA medium. The fungus *P. palmarum* was inoculated in the centre of the agar medium and was incubated at 25° C. The growth of the fungus colony was measured by recording the diameter of the colony after 24 hr incubation.

RESULTS

Data on the effect of *P. flava* extract on the spore germination of the fungus *P. palmarum* are given in Table 1. It is seen from the Table that there was a total inhibition of spore germination at 0.5% and above concentrations of the extract. However, when the concentration was reduced below 0.5% concentration of the extract there was a marginal increase in the percentage of spore germination. Malformations were also observed in the germ tube formation. Statistical analysis were carried out to assess the hypothesis whether decreasing concentrations of the extract will increase the percentage of spore germination. A correlation coefficient r = -0.2981 suggested that the hypothesis may be rejected. It is infered that the extract of *P. flava* has a pronounced inhibitory effect on the spore germination.

TABLE 1. *Germination assay : Effect of the extract of* P. flava *on the spore germination of the fungus* P. palmarum

Concentration	Percentage germination
200	0
100	0
50	0
25	0
12	0
6	0
3	0
1	0
0.5	0
0.1	10
0.01	18.2
0.001	37.6
0.0001	23.3
Control	69.7

Correlation coefficient - r = -0.2981

The effect of the extract on the mycelial growth of the fungus is given in Table 2. It is evident from the results that the inhibition of mycelial growth was directly proportional to the strength of the extract. It is interesting to note that the level of significance on the 2nd day of incubation increased with the increase of the concentration. For example, result of 25% of the extract was significant at 0.05 confidence level with the control, whereas 100% of the extract, with the control was highly significant at 0.025 level (Table 2, 2nd day of incubation). On the 3rd, 4th and 7th day of incubation there was no significant differences between the fungal growth of the control experiments and those challenged with 25% and 50% of the extract. In the case of the medium treated with 100% concentration of the extract, significant differences were seen (Table 2) when compared with control experiments. On the 3rd and 7th day of incubation significant differences were maintained.

A few additional experiments were carried out with filter sterilized extract of *P. flava*. The extract was filtered through a millipore size of 0.45 μ in order to secure the bioactive compounds in the dissolved state without suspended particles. The experimental procedures were the same as stated above.

The inhibition zone was observed after 24 hours incubation of the fungus *P. palmarum*. The results are summarised in Table 3. The intensity of inhibition was noted and represented with a symbol +. It can be seen that the intensity of inhibition decreased as the concentration was decreased. There was no significant difference in the degree of inhibition between the crude extract and the filter sterilized extract of *P. flava*.

TABLE 3. *Intensity of inhibition in relation to the concentration of the extract of* P. flava. *Filter sterlized extract*

	Concentration of the extract (%)						
	100	50	25	12	6	3	1
Intensity of inhibition	+++	+	+	0	0	0	0

+ indicates the intensity of inhibition
0 indicates no inhibition.

DISCUSSION

The results reported in the present study provide suggestive evidence that the extract of *P. flava* contains pharmacologically active biodynamic compounds. The crude extract of *P. flava* brought about a total inhibition of conidial germination at 0.5% concentration and above. A point of difference observed between the spore germination and mycelial growth of the fungus when challenged with the extract of *P. flava* is that although the spore germination was inhibited at 0.5% and above concentration, similar inhibitory effects were not observed

TABLE 2. *Diameter of the fungal colony* (P. palmarum) *when challenged with the extract of* P. flava

Incubation days	Control	Concentration of extract		
		25%	50%	100%
2nd	2.50 + 0.283	2.00 + 0.141 *	1.83 + 0.082 **	1.40 + 0.245 ***
3rd	3.50 + 0.374	2.96 + 0.082	3.06 + 0.082	2.40 + 0.245 ***
4th	4.00 + 0.374	3.65 + 0.189	3.65 + 0.074	2.63 + 0.192 ***
7th	6.60 + 0.374	6.23 + 0.356	5.80 + 0.748	3.10 + 0.283

Significance level : * - 0.05, ** - 0.025, *** - 0.005

when fungal mycelium was challenged with 25, 50 and 100% concentration of the extract. It has been reported that differences in methods of experimentation may evoke differential response of the fungus to an antimicrobial compound. In this connection, it is of interest to point out the work of Leao and Furkado (1950) who have reported that the consistency of the medium influenced the toxic action of phenols. They have reported that in liquid medium, the concentration of phenol required to inhibit the fungi *Epiphyton flucosum, Microsporum canis, Trichophyton mentagrophytes* and *T. schonleinii* was six times lesser than that required in the solid medium. In the light of the above report, it is likely that the potency of the active factor in the extract of *P. flava* may also change with culture methods. It may be worthwhile to study further the influence of the physical state of the medium on the potency of bioactive compounds.

It is known that micro-organisms may utilise the bioactive compounds as a source of carbon and oxidize them to their corresponding fatty acids during growth. On the second day after planting the mycelium of the fungus, it has been observed that the level of statistical significance increased with an increase in the concentration of the extract of *P. flava*. On succeeding days, there was no significant difference between the fungal growth in control experiments and in experimental media containing 25% and 50% of the concentration of the extract. Even on the 7th day, significant result was obtained only with the 100% concentration of the extract. These results may suggest that the active factor of the extract may be utilized for the growth of the fungus or the fungus may develop a resistence during the 3rd day of incubation when the concentration of the extract is kept below 50%.

It has been found that the halophenols of marine animals exhibit antifungal and antibacterial activities. The active factor isolated from the sponge *Dysidea herbacea* contain several antibacterial compounds which are brominated products of 2 phenoxey phenol (Sharma *et al.*, 1970). The extract of a number of echinoderms when tested against the fungus *Candida albicans* and *Saccharomycetes cervisiae*, exhibited antifungal effect (DerMardarosian, 1969, 1970). It is interesting to point out that the extract of various echinoderms elucidate differential inhibitory effects in that the amount of active factor required to produce an inhibitory effect varied with the species. The extract from *Astichopus multifidus* was found to be more effective against the fungi *Candida albicans* and *Saccharomycetes cervisiae* at 100 and 50 ppm respectively. The results of the present study indicate that the effect of extract of *P. flava*, as an antifungal agent is more acute and comparable in its effects to the extract from the echinoderm *A. multipidus*. The observed malformations in the pattern of hyphal morphogenesis during spore germination were as follows : (1) arrested germination and swelling of the spores (2) shortening of the germ tube length (3) widening of the width of the germ tube and (4) beaded germ tubes. These changes may be due to the presence of bioactive compounds in the extract of *P. flava*. It is likely that some of the bromophenols may be responsible for the antifungal properties present in the extract of *P. flava* (Higa and Scheuer, 1976; Azariah and Jeyakumar, 1988). It has been found in our investigations that fine chemicals such as 2,4,6 tribromo phenol and 2,6 dibromo phenols are also able to penetrate and inhibit the spore germination of *P. palmarum* besides inducing malformation of the germ tubes.

REFERENCES

AZARIAH, J., V. N. R. RAO AND V. T. SRIDHARAN 1978. Effect of the epidermal secretions of the Hemichordate *Ptychodera flava* on the growth of *Amphora coffeaformis* and *Cyclotella meneghiniana* (Diatoms). *Proc. Ind. Acad. Sci.*, **87** B : 145-148.

——— AND S. B. PILLAI 1985. The role of hemichordate *Ptychodera flava* in the preservation of species diversity in the Gulf of Mannar. *Proceedings of the Symposium on Marine animals and Marine parks*, MBAI, Cochin, pp. 389-400.

——— AND N. JEYAKUMAR 1988. Bioactive compounds of the marine hemichordate *Ptychodera flava* of the Madras Coast. *Proc. Symp. Tropical Marine Living Resources*, 12-16 January, 1988, Cochin, India.

DERMARDERSIAN, A. H. 1969. Marine pharmaceuticals. *J. Pharmacol.*, **58** 1-33.

——— 1970. Drugs from the sea - an overview. *In* : H. W. Youngken Jr. (Ed.) *Food drugs from the sea.* Marine Technology Society, Washington, 211-253 pp.

HIGA, T. AND P. J. SCHEURE 1976. Bisindoxyl derived blue marine pigments. *Heterocycles*, **4** : 227-253.

HUMN, H. J. AND C. E. LANE 1974. Bioactive compounds from the sea. Marcel and Dekker Inc., New York, pp. 295-305.

LEAO, A. E. AND A. D. R. FURTADO 1950. The fungistatic activities of Vitamin K on dermatophytes. *Mycopath Micol. Appl.*, **5** : 121-124.

RICKER, A. J. AND R. S. RICKER 1936. *Introduction to Research on plant diseases.* John S. Swift & C, St. Louis.

SHARMA, G. M. AND P. R. BURKHOLDER 1967. Studies on the antimicrobial substances of sponge II. Structure and synthesis of bromine containing antibacterial compounds from marine sponge *Verongia cauliformis. Tetrahedron lett.*, **42** : 4147-4150.

SHARVELLE, E. G. 1961. The nature and use of modern fungicides. Burges Publishing and Co., Minneapolis, Mlmu.

SHIMADA, S. 1969. Antifungal steroid glycoside from sea-cucumber. *Science*, **163** : 14-62.

SWAMY, R. N. AND K. MANI 1978. Sporulation of *Pestalotia palmarum* Cook in culture. *Proc. India. Acad. Sci.*, **87B** : 93-98.