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INVITRO SCREENING OF ANTIFUNGAL POTENTIAL OF CRUDE EXTRACTS OF THE MARINE GASTROPOD, *TURBINELLA PYRUM*

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ABSTRACT

The aim of the study was to determine the effect of different solvent extracts of soft tissue extracts of *Turbinella pyrum* against five fungal strains *viz Fusiarium oxysporum*, *Aspergillus niger*, *A.flavus*, *Candida albicans* and *Mucor sp*. The antifungal activity of the sample was tested by disc diffusion technique using PDA (Potato Dextrose Agar) medium. The highest inhibition zones were developed by methanol extract against all the tested fungal strains and lowest activity was observed in ethanol extract against all fungal pathogens. The crude solvents of the marine gastropod *T.pyrum* were found to be promising source of antifungal agents and exhibits remarkable activity against all the 5 fungal strains.

Keywords: Fungal strains, *Turbinella pyrum*, Disc diffusion, PDA medium

INTRODUCTION

Fungal diseases are a major medical problem worldwide. These diseases are troublesome than ever before. The therapy for most invasive fungal diseases remain unsatisfactory given their morbidity and mortality despite the available antifungal treatment. The nature is the store house of natural products with development of science and technology. New discoveries have shifted attention from synthetic model and compounds to natural products of animal origin **[1]**. Molluscs hold great potential for development as a source of therapeutically useful compounds. A number of marine molluscs are also used in traditional Chinese, Indian, South African &

middle Eastern medicines as well as in homeopathic remedies **[2]**.

Around 52% of the natural compounds identified from molluscan origin were not analyzed for any kind of biological activities, whereas less than 1% of identified molluscan species were studied for the presence of bioactive secondary metabolites. More recently, the molluscan products and their synthetically formulated structural analogs were recognized in the clinical trials of various diseases **[3]**.

The treatments with currently used antifungal agents require long term administration protocols capable of causing toxic. The standard antifungal therapies can be also limited because of low efficacy rates and drug resistance. Despite involvement of antifungal therapies during the recent 20 years, the phenomenon of antifungal resistance is still a mayor concern in clinical practices **[4, 5]**. Therefore, the snails should be considered important bioactive compound sources with safe pharmaceutical applications.

A review of literature on antifungal activity of molluscs particularly gastropod revealed that only a sketchy information is available. Prem Anand and Patterson Edward **[6]** (2002) examined the antifungal activities of five species of *Cypraea*. Antifungal activity of tissue extract of *Babylonia spirata* was reported by Periasamy **[7]** *et al.*,2012, Umaya Parvathi[8] *et al.,*2012, studied the antifungal activity of many gastropods *viz*, *X.pyrum*, *Turitella acutangula*, *Hemifuses cochlidium*, *Bursa rana, B.crumena*, *Rapana rapiformis* and *Ficus ficus*. Harekrishna jana **[9]** *et al.*, 2017 on *Bellamya bengalensis*, Kanagasabapathy **[10]** *et al.*, (2011) on *Melo melo*, Ulagesan and Kim **[11]** (2018) observed the antifungal activities of seven different snails. Krumova **[5]** *et al.*, (2021) evaluated the antifungal activity of separated fractions from *Rapana venosa*. Antifungal activity of soft tissue extract of garden snail *Helix aspera* was determined by Azeem **[12]** *et al*., (2022). Hence the present study was designed to determine the effect of different solvent extracts of *T.pyrum* against 5 different fungal strains *viz*., *Fusiarium oxysporum*, *Aspergillus niger*, *A.flavus*, *Candida albicans* and *Mucor sp*.

MATERIAL & METHODS

Collection and extraction of samples: Live specimens of *T.pyrum* were collected from Gulf of Mannar coastal region of Thoothukudi, from the trawl nets used for capturing crabs. They were immediately brought to the laboratory and their soft bodies were removed by breaking the shells. The whole body muscle of the sample (50g) was cut into small pieces and the tissue sample was used for extraction by using different solvents such as ethanol, methanol, acetone and chloroform. The extracts were cold seeped overnight at -18°C and filtered

with Whatman No.1 filter paper. The filtrate was poured in previously weighed petriplate and evaporated to dryness in rotary evaporator (Becerno *et al*, 1994 and Wright 1998). The dried extracts were used for antifungal assay against 5 fungal pathogens *viz*., *Fusarium oxysporum*, *Aspergillus niger*, *A.flavus*, *Candida albicans* and *Mucor sp* & all these fungal strains were obtained from Raja Muthiah Medical College, Annamalai University.

Assay of antifungal activity/ Anti-Fungal Screening:-

The antifungal activity of the sample was tested by disc diffusion technique using PDA (Potato Dextrose Agar) medium against the following pathogens *Fusiarium oxysporum*, *A.niger*, *A.flavus*, *C.albicans* and *Mucor sp*. The fungicide nyastin was used as positive control.

A sterile paper disc (5mm) impregnated with test samples (50ml /disc) was prepared by dissolving it with DMSO. The PDA plates were swabbed with *F.oxysporum, A.niger, A.flavus, C.albicans* and *Mucor sp* and the sterile disc impregnated with sample was placed on the plate, incubated at 28°C for 48-72 hours. The nyastin standard discs were used as a positive control and the disc impregnated in PBS is used as a negative control. The zone of inhibition (mm) was read after 72 hours of incubation (clear zone of inhibition formed around were considered indicative of antifungal activity). Each extract form was evaluated with three kept repetitions (Bacero *et al*, 1994, Wright 1998 and National Committee for clinical laboratory Standards, 2002).

RESULTS

Totally four extracts from the gastropod *T.pyrum* were screened against 5 fungal pathogens. The inhibition zones developed by ethanol, acetone, chloroform and methanol extracts against test organisms were given in **Table 1 and Figure 1**. Of the four extracts tested here methanolic extract showed more potent activity by developing inhibition zones of 19mm against *C.albicans*, 17 mm against *A.niger*, 15mm against *F.oxysporum*, 14 mm against *A.flavus* and 10 mm against *Mucor sp*. The acetone and chloroform extracts developed maximum zone (12mm) against *F.oxysporum* and minimum of 5 mm & 8mm respectively against *Mucor sp*. Ethanol extract was able to produce a zone of 9mm against *F.oxysporum,* 5mm against *C.albicans*, 4mm in *A.niger,* 3mm in *A.flavus* and 2 mm in *Mucor sp*. The more significant growth inhibition was observed for *C.albicans* against all the four extracts (inhibition zones of 10 mm in ethanol and acetone, 11 mm in chloroform and 19mm in methanol). Next to *C.albicans*, *F.oxysporum* also developed maximum inhibition zones viz., 9mm, 12mm, 12 mm and 15 mm in ethanol, acetone, chloroform & methanol

extracts respectively. Of the 4 extracts tested here methanol showed more potent activity followed by chloroform, acetone and ethanol.

Table 1: The quantum of antifungal activity of crude solvent extracts of *T.pyrum* against fungal pathogens

Fungal strains	Ethanol	Acetone	Chloroform	Methanol	Nystatin	PBS Negative control
F.oxysporum	**9**	**12**	**12**	**15**	**28**	**-**
A.niger	**4**	**6**	**7**	**17**	**-**	**-**
A.flavus	**3**	**8**	**9**	**14**	**-**	**-**
C.albicans	**10**	**10**	**11**	**19**	**-**	**-**
Mucor sp	**2**	**5**	**8**	**10**	**-**	**-**

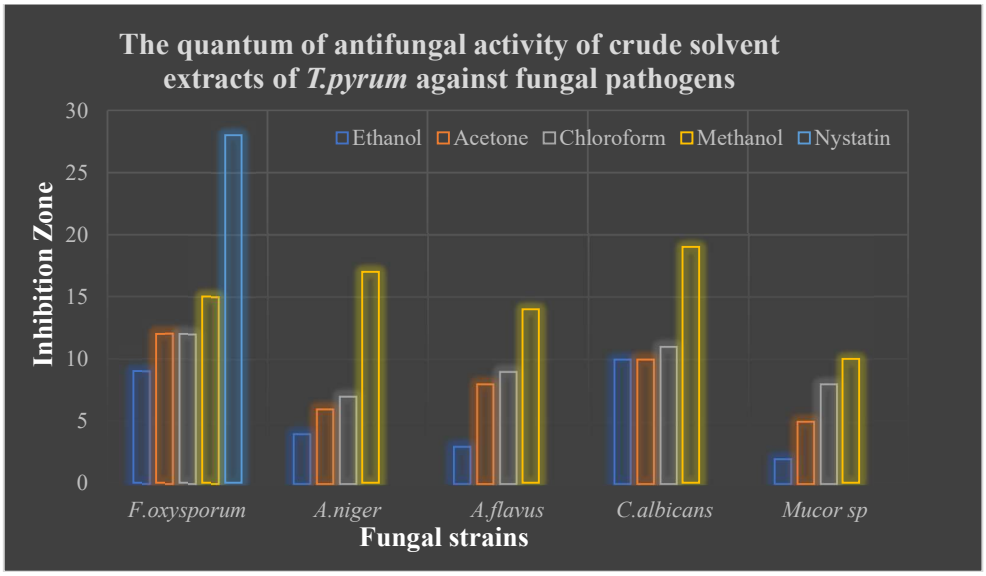

Figure 1: The quantum of antifungal activity of crude solvent extracts of *T.pyrum* against fungal pathogens

DISCUSSION

Marine organisms, especially molluscs have found to produce a great diversity of novel bioactive secondary metabolites, the potential source for new drug discovery. In the industrialized countries, about 25% of all prescription drugs contain active principles that are extracted from higher plants. In traditional Indian medicine, especially Siddha Medical preparations the opercula of gastropods are used as ingredient to combat different diseases **[7]**. In the present study highest inhibition zones were developed by methanol extract against all the tested fungal strains and lowest activity was observed in ethanol extract against all fungal pathogens. Umayaparvathy **[8]** *et al.,* (2012) studied the antifungal activity of 10 marine gastropods, of them the methanol extract of *B.rana* showed maximum antifungal activity by developing inhibition zones of 12 mm, 8mm, 5mm and 9mm *Trichoderma sp*, *Trichothecium sp* and *Hormodendrum sp* and *Rhizopus sp* respectively. The crude methanolic extract of *Cypraea errones* exhibited antifungal activity against *A.niger* and *C.albicans* **[8]**. Bhosale **[13]** *et al*, (1999) reported the strong antifungal

activity of marine molluscs such as *Elysia grandifolia* against 3 fungal strains such as *A.fresenii*, *A.japonicus* & *A.niger*. The solvent extract of coral associated gastropod *Trochus tentorium* showed potent antifungal activity against human pathogens **[14]**. The Saline extract of *M.meretrix* and *M.casta* showed maximum zone of clearance against *A.fumigatus*, *A.flavers* & *C.albicans*. The extract of marine mollusc *Melo melo* exhibited maximum antifungal activity against *Trychophyton metagerophytes* and minimum activity against *A.flaves* **[10]**. All the above reports corroborating the results of the present investigation. *À.niger* was the most sensitive (12.09±0.06mm) against ethyl acetyl extracts of *T.tissoti* and *C.albicans* exhibited high zone (8.13±0.15mm) against n-butanol extract of *B.spirata* (Periasamy *et al*, 2012). The results of the present study agrees well with the above findings.

In fungal assay by Suresh **[15]** *et al*., 2012, *C.albicans* showed high susceptibility (7.13mm zone of inhibition) against methanol extract of *B.zeylonica*, lowest inhibition zone (1mm) was noticed by *A.flavus* against ethanol extract and also observed that the *H.conoidales* developed the highest inhibition zone (4.03 mm) by *A.niger* against ethanol extract & lowest inhibition zone (1mm) against *C.albicans* and *Mucor sp* against the same extract. These results lend support to the antifungal activity of 4 extracts of *Turbinella pyrum*. Santhi **[16]** *et al.* (2016) also found out the same result at 100mg/ml concentrations of solvents. These studies agree well with the result of the present finding. In the present study crude solvents of the marine gastropod *T.pyrum* were found to be promising source of antifungal agents and exhibits remarkable activity against all the 5 fungal strains. Further studies on isolation and characterization of the compounds which could be responsible for antifungal study are required.

REFERENCES

[1] Thilaga, RD (2005). 'Studies on some ecological aspects of Babylonia spirata (Linn) along the Tuticorin coast', Doctoral dissertation, Manonmaniam Sundaranar University.

[2] HPUS. (1878) Homeopathic Pharmacopoeia of the United States, 1st ed.; Chicago Duncan Brothers Publishers: Chicago, IL, USA, 1878.

[3] Simmons, TL. Andrianasolo, E. McPhail, K. Flatt, P. and Gerwick, WH. (2005) Marine natural products as anticancer drugs. *Mol. Can. Ther. 4*, 333–342.

[4] Wiederhold NP. (2017) Antifungal resistance: current trends and future strategies to combat. Infect Drug Resist.10:249-259.

[5] Krumova, E., Dolashka, P., Abrashev, R., Velkova, L., Dolashki, A., Daskalova, A., & Angelova, M. (2021). Antifungal activity of separated fractions from the hemolymph of marine snail *Rapana venosa*. *Bulg. Chem. Commun*, *53*, 42-48.

[6] Anand, T. & Patterson, Edward. (2002). Antimicrobial activity in the tissue extracts of five species of cowries *Cypraea spp*. (Mollusca: Gastropoda) and an ascidian *Didemnum psammathodes* (Tunicata: Didemnidae). *Indian J Mar Sci*. 31 (3)

[7] Periyasamy N, Srinivasan M, Balakrishnan S. (2012) Antimicrobial activities of the tissue extracts of *Babylonia spirata* Linnaeus, 1758 (Mollusca: Gastropoda) from Thazhanguda, southeast coast of India. Asian Pac J Trop Biomed. 2(1): 36-40.

[8] Umayaparvathi, S., Arumugam, M., Meenakshi, S., and Balasubramanian, T. (2012). Studies on antifungal, cytotoxic activities of molluscs and echinoderm extracts from southeast coast of India. Asian J. Pharm. Biol. Res. 2, 198–203.

[9] Harekrishna Jana, Abhijit Mitra and Keshab Chandra Mondal. (2017); Comparative Study on the Antimicrobial activity, Anti-oxidant, Anti-Diabetic and Anti-Inflammatory property of Fresh Water Molluscs (Bellamya Bengalensis) and marine molluscs (Saccostrea Cucullata.) *Int. J. Of Adv. Res.* **5**. 16-23.

[10] Kanagasabapathy & Samuthirapandian, Ravichandran & Kumaresan, M. (2011). Preliminary studies for a new antibiotic from the marine mollusk Melo melo (Lightfoot, 1786). Asian Pacific journal of tropical medicine. 4, 310-4.

[11] Ulagesan, S., & Kim, H. J. (2018). Antibacterial and antifungal activities of proteins extracted from seven different snails. *Applied Sciences*, *8*(8), 1362.

[12] Azeem, H. H. A. E., Osman, G. Y., El-Seedi, H. R., Fallatah, A. M., Khalifa, S. A., & Gharib, M. M. (2022). Antifungal Activity of Soft Tissue Extract from the Garden Snail Helix aspersa (Gastropoda, Mollusca). *Molecules*, *27*(10), 3170.

[13] Bhosale, S. H., Jagtap, T. G., & Naik, C. G. (1999). Antifungal activity of some marine organisms from India, against food spoilage Aspergillus

strains. *Mycopathologia*, *147*, 133-138.

[14] Anbuselvi, S., C.Chellaram, S. Jonesh, A. Jayanthi and J.K.P. Edward.2009. Bioactive potential of coral associated gastropod, *Trochus tentorium* of Gulf of Mannar, southeastern India. Journal of Medicinal Science 9:240-244.

[15] Suresh, M., Arularasan, S., & Srikumaran, N. (2012). Screening on antimicrobial activity of marine gastropods Babylonia zeylanica (Bruguière, 1789) and Harpa conoidalis (Lamarck, 1822) from Mudasalodai, southeast coast of India. *Int J Pharm Pharm Sci*, *4*(4), 552-556.

[16] Santhi, V., Sivakumar, V., Jayalakshmi, S., Thilaga, R. D., & Mukilarasi, M. (2016). Isolating Bioactive Compound from Marine Prosobranch Purpura persica from Tuticorin Coast. *International Journal of Environmental Protection and Policy*, *4*, 64-76.