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N Peetambari Devi
a) Kerala University of Fisheries and Ocean Studies, Panangad Road, P.O. Kochi, Kerala, India
b) ICAR- Research Complex for NEH Region, Umiam, Meghalaya, India

J Rajashekaran Nair
Kerala University of Fisheries and Ocean Studies, Panangad Road, P.O. Kochi, Kerala, India

KV Jayachandran
Kerala University of Fisheries and Ocean Studies, Panangad Road, P.O. Kochi, Kerala, India

Devika Pillai
Kerala University of Fisheries and Ocean Studies, Panangad Road, P.O. Kochi, Kerala, India

Alphy Korath
Kerala University of Fisheries and Ocean Studies, Panangad Road, P.O. Kochi, Kerala, India

Nongmaithem Raju Singh
ICAR- Research Complex for Eastern Region, Patna, Bihar, India

Corresponding Author:
N Peetambari Devi
a) Kerala University of Fisheries and Ocean Studies, Panangad Road, P.O. Kochi, Kerala, India
b) ICAR- Research Complex for NEH Region, Umiam, Meghalaya, India

Screening of antifungal activity of accessory nidamental glands (ANGs) and nidamental glands (NGs) of *Uroteuthis singhalensis* against selected microbes

N Peetambari Devi, J Rajashekaran Nair, KV Jayachandran, Devika Pillai, Alphy Korath and Nongmaithem Raju Singh

Abstract
The present investigation demonstrates the antifungal activity of accessory nidamental glands (ANGs) and nidamental glands (NGs) of *Uroteuthis singhalensis* against selected microbe's *viz*., *Aspergillus flavus, A. fumigatus, Candida albicans* and *Fusarium solani*. The mature ripe female squid NGs and ANGs were collected and extracted by using two solvents namely ethanol and butanol. Disc diffusion technique was employed to determine the antifungal property of different extracts. The antibiotic Amphotericin-b was taken as positive control. It was found that the use of different solvent for extraction on expression of antifungal activity by different fungal strains found to be significant varied. The highest antifungal activity in ANGs extract by using ethanol (12 ±0.47 mm) and butanol (15±0.39 mm) solvents was achieved against *Aspergillus fumigatus* and *Aspergillus flavus* respectively. Similar result was also found in NGs extracts. Interestingly, the different extracts did not show any antifungal activity against the fungal strain *Fusarium solani.* Overall, the results showed that butanol solvent extraction had exhibited greater inhibitory effect as compared to ethanol solvent extraction.

Keywords: Antifungal activity, nidamental glands (NGs), accessory nidamental glands (ANGs), *Uroteuthis singhalensis*

1. Introduction
Bio-active compounds of high importance value are constantly derived from the diverse marine ecosystem. In this aspect, cephalopod has been recognized as an important marine organism. Squid represent an important member of cephalopods under phylum mollucans which has enormous contribution as food sources and pharmaceutical substances (Hoque *et al*., 2010) [1]. The role of NG and ANG in forming the egg sheaths and their role in the protection of eggs and embryos in squids have also been well documented (Biggs and Epel, 1991) [2]. The disease occurrence on marine organisms brought by several fungal species has created a big economical loss in marine production. This problem has again exaggerated by present changing climate issues and multi resistant microbial strains found inside marine water body. In this context, large cache of work on the antibacterial activity of ANG has conducted (Grigioni *et al*., 2000; Sherief *et al*., 2004; Gomathi *et al*., 2010) [3,4,5]. However, few limited studies have been conducted so far on the aspect of antifungal activity of *Uroteuthis singhalensis*. A study was carried out with an aim to unearth the potentiality of Indian squid as an important antifungal agent.

2. Materials and methods
2.1 Collection of accessory nidamental glands (ANGs) and nidamental glands (NGs)
Collection of visceral mass from mature ripe female of squid (*Uroteuthis singhalensis*) was collected from Mangala's Aquatic Products, Aroor, Alleppey District, Kerala, Bhatson's Aquatic Products, Aroor, Alleppey District, Kerala and Torry Harris Seafoods (Pvt.) Ltd., Eramalloor, Alleppey, Kerala was done during the peak season of maturity. The ANGs of mature female squid appear red or mottled red in colour and the extract is reddish in colour while NGs appear creamy white in colour and the extract is colourless.

2.2 Preparation of extracts

Exhaustive method was applied for extraction of the glands in ethanol and butanol medium. Extraction was done by using homogenized ripe glands tissue of 2.5 g with 5.0 ml butanol and ethanol. The extraction has become completed when the extracts becomes colourless. The extract was concentrated by passing nitrogen gas over it. The extract was stored at -20 °C for further antifungal assay (Sherief *et al*., 2004) [4, 15].

2.3 Selection and culturing of fungal strains

The four fungal culture strains used in this study are *Aspergillus flavus* (MTCC no. 2206) *A. fumigatus* (MTCC no. 6500), *Candida albicans* (MTCC no. 7253) and *Fusarium solani* (MTCC no. 6343). The freeze dried culture strains were sub cultured in the prescribed medium and temperature as per the protocol of the Microbial Type Culture Collection (MTCC).

Table1: Details about the fungal culture (as instructed by MTCC)

Species	Growth medium	Growth condition	Growth temperature	Incubation time	Subculture time
Candida albicans	Malt Yeast Agar	Aerobic	25 °C	2 days	3 months
Aspergillus flavus	Czapek Yeast Extact Agar	Aerobic	25 °C	5 days	1 month
Aspergillus fumigatus	Czapek Yeast Extact Agar	Aerobic	25 °C	5 days	1 month
Fusarium solani	Potato Dextrose Agar	Aerobic	25 °C	5 days	1 month

2.4 Antifungal activity assessment

The antifungal activity of ripe female of squid accessory nidamental glands (ANGs) and nidamental glands (NGs) was screened by using filter paper disc diffusion technique and the antifungal activity was presented in accordance with the level of inhibition zone in mm (Collins and Lyne, 1967) [6].

2.5 Statistical Analysis

Data obtained from each parameter were expressed in Mean and standard error of mean (S.D). Data were subjected to perform one way analysis, Duncan's multiple range test (DMRT) at $p < 0.05$ by using SPSS 17.0 (SPSS Inc., Chicago, USA) windows version package.

3. Results

The assessment of antifungal activity of mature ripe ANGs and NGs extracts from the *Uroteuthis singhalensis* was studied against four selected fungal species *viz*., *Candida albicans, Aspergillus flavus, Aspergillus fumigatus and Fusarium solani* (Table 2 and 3). The result obtained from ANGs extract had shown that the use of different solvent for extraction on expression of antifungal activity by different fungal strains found to be significant varied (Table 2). The data also revealed that there was significant variation among the fungal strains on the performance of their antifungal activity. Under, the ethanol solvent extraction, the highest (12 ±0.47mm) antifungal activity was shown against *Aspergillus fumigatus* which is also greater than the results produced by positive control Amphotericin – b (11±0.42mm) however; there was no antifungal activity against *Aspergillus flavus* and *Fusarium solani*. Contrarily, in case of butanol solvent extraction the highest (15±0.39) antifungal activity was achieved against *Aspergillus flavus* followed by *Aspergillus fumigatus* (13±0.33mm) which is also higher than the positive control (Amphotericin – b) while the extracts did not attained any antifungal activity against *Fusarium solani*. Overall, results found that butanol solvent extraction had exhibited greater inhibitory effect as compared to ethanol solvent extraction (Figure 1).

The ethanol solvent extraction results obtained from NGs showed similar result that of ANGs extract. While, in case of butanol solvent extraction, the ANGs extract produce similar range of antifungal activity in all the fungal strains except *Fusarium solani* where, it showed no antifungal activity. Similarly with ANGs extract, NG extract also produced higher antifungal activity in butanol solvent extraction as compared to ethanol solvent extraction (Table3). Interestingly, the extracts performed under butanol condition had better antifungal inhibition than positive control Amphotericin – b except the *Fusarium solani*.

Table 2: Mature female squid (*Uroteuthis singhalensis*) ANG extracts and their antifungal action against selected four fungal species

Species	ANG					
	Amphotericinb	SEm	Ethanol	SEm	Butanol	SEm
Aspergillus flavus	12^{a}	±0.47	0^{c}	±0.00	15^{a}	±0.39
Aspergillus fumigatus	11^{ab}	±0.42	12^{a}	±0.37	13^{b}	±0.33
Candida albicans	10^{b}	±0.33	10^{b}	±0.39	10^{c}	±0.39
Fusarium solani	10^{b}	±0.37	0^{c}	±0.00	0^{c}	±0.00

Values followed by different alphabets in parenthesis are significantly different at $p < 0.05$ based on Duncan's multiple range test (DMRT).

Table 3: Mature female squid (*Uroteuthis singhalensis*) NG extracts and their antifungal action against selected four fungal species

Species	NG					
	Amphotericin – b	SEm	Ethanol	SEm	Butanol	SEm
Aspergillus flavus	12^{a}	±0.44	0^{c}	±0.00	15^{a}	±0.39
Aspergillus fumigatus	11^{ab}	±0.39	12^{a}	±0.47	14^{a}	±0.36
Candida albicans	10^{b}	±0.33	10^{b}	±0.36	15^{a}	±0.39
Fusarium solani	10^{b}	±0.29	0^{c}	±0.00	0^{b}	±0.00

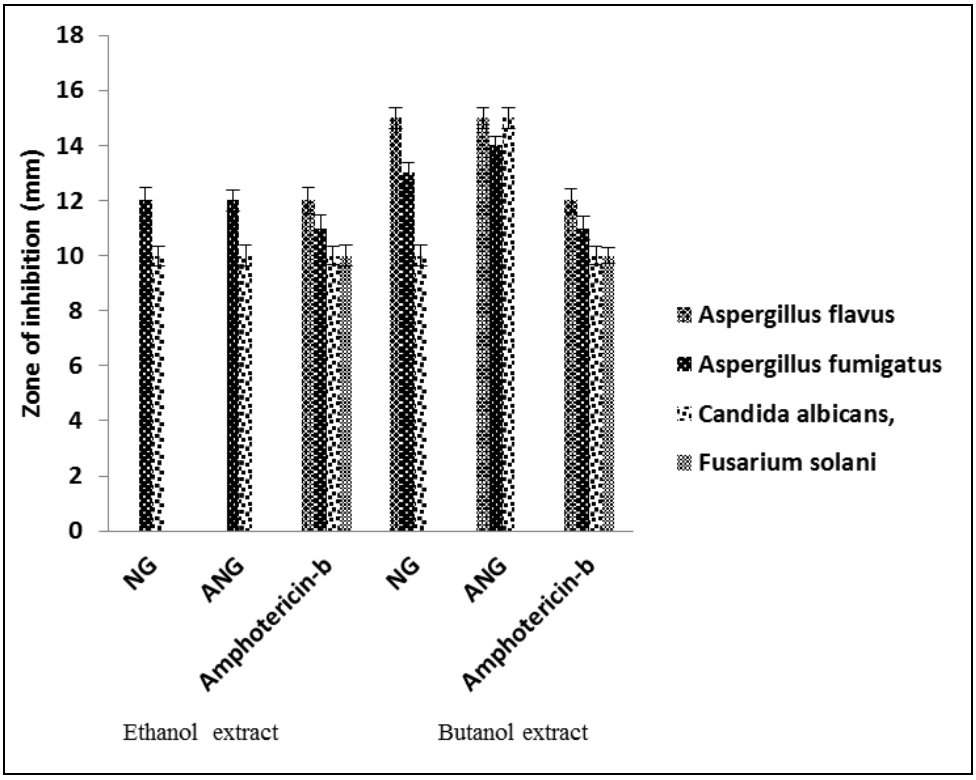

Fig 1: Antifungal activity of ethanol and butanol ANGs and NGs extracts of *Uroteuthis singhalensis.*

4. Discussion

The present study results had shown that there was significant influence of different solvent used for extraction on expression of antifungal activity by different fungal strains and this results is in agreement with Ismail and Riad R (2018) [7]. The butanol solvent extraction had produced better inhibitory result as compared to ethanol solvent extraction (Venkatesan *et al*., 2014) [8]. Benkendroff *et al.* (2001) [9] had also found that butanol extract produced highest antibacterial activity of marine mollucus followed by methanol extraction. The results from the works of Ismail and Riad (2018) [7, 16] and Vasantharaja *et al*., (2014) [10] had reported that methanol extraction produced highest antimicrobial activity than other solvent used however there was no used of butanol solvent extraction in their studies. In this present study, the performances of different fungal strain showed variation where *Aspergillus fumigatus* showed the highest (12±0.44 mm) antifungal activity in case of ethanol solvent extraction while *Aspergillus flavus* produced highest inhibitory result in butanol condition. Unfortunately, *Fusarium solani* did not showed any antifungal activity in both ethanol and butanol extraction condition which is might due to absence of antifungal compounds. The previous work carried out by Barwin Vino (2003) [11] had reported that polysaccharide extract from the gladius of *Loligo duvauceli* exhibited antifungal action against *Aspergillus fumigatus*, *A. flavus* and *Rhizopus* sp. Shanmugam *et al*. (2008) [12] also reported that a squid, *Euprymna berryi* have produced antifungal activity against *Candida albicans, C. neofromans* and *Aspergillus fumigatus*. On the account of comparison between the ANGs and AGs extract there was more or less similar results was achieved however, NGs extract recorded some extend of better results of *Aspergillus fumigatus* and *Candida albicans* in butanol solvent extraction condition. Sherief *et al*. (2004) [4, 17] had reported that ANG-butanol extracts of *Sepia aculeata*, *S. pharaonis*, *Sepiella inermis* and *Loligo duvauceli* possess antibacterial property where *S. aculeata* produced the highest antibacterial activity. Sherief *et al.* (2007) [13] studied the free fatty acid composition of the ripe ANG of *Sepia pharaonis* of the Kochi region. The four major fatty acids were Palmitic acid (hexadecanoic acid) (15.013%), followed by stearic acid (octadecanoic acid) (10.506%), and unsaturated fatty acids like docosahexaenoic acid (DHA) (8.180%) and eicosapentaenoic acid (EPA) (8.102%). The antibacterial activity of the ANG extracts increased with the colour intensity and reciprocal increase in the free fatty acid contents of the ANG. Gomathi (2008) [14] reported that four major fatty acid components of the ANG of *Uroteuthis singhalensis* from Kochi, as palmitic acid at 0.867mg/g tissue, followed by the unsaturated fatty acids like docosahexaenoic acid (DHA) at 0.680 mg/g tissue, oleic acid at 0.657 mg/g tissue and eicosapentaenoic acid (EPA) at 0.305 mg/g tissue and these attributed the antibacterial property of the squid ANG-butanol extract.

5. Conclusion

The ANGs and NGs extracts of *Uroteuthis singhalensis* in different solvent extraction condition *viz*., methanol and butanol holds fungicidal properties. The butanol solvent extraction exerts more antifungal activity as compared to methanol solvent extraction. The ANGs and NGs extracts of *Aspergillus flavus*, *Aspergillus fumigatus* and *Candida albicans* can be used as an antifungal agent. Thus, the present study has immense role in generating the baseline information for identification of natural origin substance for future pharmaceutical use.

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