# Antimicrobial and phytochemical evaluation of ascidian Phallusia nigra from Vizhinjam Bay, Southwest coast of India

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***Abstract-*** *The antimicrobial activity of methanol, ethanol, chloroform, aqueous and ethyl acetate were carried out by using disc diffusion method against various pathogenic organisms. The crude ethanol extract was more active exhibiting a broad spectrum of antibacterial activity. Maximum Inhibition zone (16mm) was observed against Staphylococcus aureous in 40µg/ml concentration of ethanol extract. The minimum inhibition zone (7mm) was observed in chloroform extract of streptococcus mutans against 40 µg/ml concentration.Among the antifungal activity maximum Inhibition zone 17mm was observed in Aspergillus flavusof ethanol extract and Minimum Inhibition (7mm) was observed in Rhizopus species of methanol and chloroform extract of 40 µg/ml concentration. The preliminary phytochemical screening of various extracts was carried out.The extractive value was Maximum in protein (ie) 120 µg/ml and 65 µg /ml in flavonoid of ethanol extract and minimum 4 µg/ml in Tannin of ethanol extract. Alkaloids, steroids, flavonoids, tannin, saponins, carbohydrates, amino acids, proteins, phenols and lipids are present. FTIR results showed that mostly phenolic compound carboxylic acids and alkane group present in ascidian Phallusia nigra.*

***Keywords***- Phallusia nigra, Antimicrobial, Antifungal, Phytochemical screening, FTIR.

## I. INTRODUCTION

The ocean is considered to be an untapped source for many things including potential drugs. A large proportion of natural compounds have been extracted from marine invertebrates especially sponges, ascidians, bryozoans and molluscs and some of them are currently used in clinical trials (Proksch et al., 2002). Ascidians are marine invertebrates which ranks second with promising the source of drugs (Azumi et al., 1990). Most of the ascidians are utilized as food in various countries and they are known to produce bioactive metabolites which prevent bio-fouling and this can be considered as a kind of autogenic protection (Barguest and Bed ford, 1978). Tunicates have been reported as rich sources of biologically active compounds and ranked third for their overall activities next to sponges and bryozoans (Davis and Bremner, 1999). These compounds are mainly comprised of various derivatives of alkaloids and peptides. There are few examples of marine derived compounds which have successfully reached the market as therapeutic drugs. A large group of low molecular weight natural compounds that exhibit antimicrobial activity has been isolated from plants and animals during the past two decades. Polyphenols and other phytochemical have been shown to have antioxidant activities, it has been suggested that consumption of polyphenol-rich foods is associated with reduced risk of cardiovascular diseases, stroke and certain types of cancer (Borros et al., 2007 and Jegadish et al., 2003).

However the phytochemical analysis using these extracts of *phallusia nigra* has not been carried out so far.

## II. MATERIALS AND METHODS

### Collection and preparation of sample

The ascidian, *Phallusia nigra* (Chordate: Ascidiacea) was collected during the low tide of the intertidal are atVizhinjam south coast of India during April 2017. The collected samples were rinsed with sterile sea water to remove associated debris and salt. The samples were weighed (10g) and preserved separately in methanol and ethanol (1:2) and brought to the laboratory

### Preparation of the extract

The test samples were carefully removed washed with sterile seawater dried under shade and homogenized to get a course powder. The course powder was stored in an air tight container and used for further investigation. 100g of the powdered animal material was extracted with methanol, ethanol, chloroform, ethyl acetate and aqueous using soxhlet apparatus. The extract was cooled to room temperature,

evaporated in a rotary evaporator under reduced pressure and a brown sticky residue was obtained (15g).

**Antibacterial assay**

The bioassay was carried out using the agar disc diffusion method (Bauretal., 1966). Muller Hinton agar plates are prepared by pouring 15ml of medium and allowed to solidify. The petriplated are swabbed with 24 hour old culture of the selected bacterial strains. The sterile paper discs were loaded with different solvents concentration and allowed to dry thoroughly. Then the discs were placed over the plates and incubated from 24hr at $37^0$C.

**Antifungal assay**

The bioassay was carried out using Ratiram et al., 2015). Zobell dextrose agar medium were prepared and the compound was allowed to diffuse for 5 minutes and the plates were kept for incubation at $37^0$c for 24 hours. At the end of incubation inhibition zones were examined.

**Phytochemical analysis**

**Qualitative analysis and Quantitative analysis**

The following phytochemicals were qualitatively and quantitative determined in the ascidian *Phallusia nigra* among the 5 different extracts.

Carbohydrates estimated by Fehling's Test (Kokate,1994),Glycosides (Ansari 2006) by Keller – Killiani test), Steroids (IP,1996) by Salkowski Test, Alkaloids (Ansari 2006) byMayers Test, Flavanoids (kokate, 1994) by Shinoda Test, Tannin (Mukherjee, 2002) by Lead acetate test, Saponin (Ansari, 2006) byFoam test, Protein (Ansari, 2006) by Biuret test, Phenol by Ferric chloride test (Mutherjee, 2002),Amino acid by Ninhydrin (Ansari 2006). Are the test used for qualitative andquantitative determination.

**FTIR**

Methanol, ethanol, chloroform, ethyl acetate and aqueous extract of *Phallusia nigra* were recorded by Fourier-Transformed Infrared Spectra (Shimadzu,UK). FTIR was used to identify the chemical structure in a wide range of compounds. Infrared spectroscopy was a useful analytical tool for detection of functional groups in organic compounds. IR spectra were recorded in the 400-4000 $cm^{-1}$ with a resolution of $km^{-1}$. The room was kept at a controlled ambient temperature ($25^o$C) and relative humidity 30% (Sayed et al., 2005).

## III. RESULTS AND DISCUSSION

The results of antibacterial activity and antifungal activity of the crude methanol, ethanol, ethyl acetate, chloroform and aqueous extract of *Phallusia nigra* against 3gram positive and 3gram negative bacteria (Table 1).

Table 1 Antibacterial and Antibacterial activity of aqueous extract from ascidian *Phallusia nigra*

| Sample Name | ANTIBACTERIAL | | | | | | ANTIFUNGAL | | |
|---|---|---|---|---|---|---|---|---|---|
| | *Staphylococcus* | *Streptococcus* | *Bacillus subtilis* | *Klebsiella* | *Proteus vulgaris* | *Escherichia coli* | *Aspergillus niger* | *Aspergillus flavus* | *Rhizopus stolonifer* |
| Ethanol | 16 | 13 | 11 | 11 | 8 | 9 | 13 | 17 | 11 |
| Methanol | 10 | 10 | 10 | 11 | 9 | 12 | 14 | 14 | 7 |
| Ethylacetate | 9 | 9 | NZ | NZ | 8 | NZ | NZ | 9 | NZ |
| Chloroform | 8 | 7 | 10 | NZ | N2 | NZ | NZ | 13 | 7 |
| Aqueous | NZ | NZ | NZ | NZ | NZ | 8 | 8 | NZ | NZ |
| Positive Control (streptomycin) | 25 | 26 | 18 | 26 | 21 | 26 | 26 | 21 | 17 |

Ethanol extract of 40ug/ml concentration produced maximum inhibition zone of 16mm against *Staphylococcusaurus* and minimum of 7mm in *Streptococcus*mutants. Corresponding zones of methanol extract produced 12mm and 11mm against *E.coli* and *Proteus vulgaris* (Table 1). All the 5 extracts of some concentration of different organisms showing minimum activity against ethylacetate and chloroform against *Staphylococcusaureus*, *Streptococcus* mutans *E.coli, B.subtilis, K.pneumonia, P. vulgaris*. This observation is consistent with the findings of Ananthan et al., (2011) who reported that both methanol and ethyl acetate extract of *P. nigra* showed a broad spectrum of antibacterial activity against tested gram negative pathogens. Antibacterial activity of ascidians extract increased with increasing concentrations. Antibacterial activity of some tunicates has been previously reported fromvarious authors of Thompson *et al*. 1983, Mohamed Hussain and Ananthan, 2009, Sivaperumal et al., 2010, Ananthan et al, 2011, Mohamed Hussain et al, 2004. The results of the present study showed that highly potent antibacterial substance is found in

*Phallusia nigra*. Antibacterial activity of crude extract of ascidian showed inhibiting activity against almost all the 6 human pathogen strain. The range of inhibiting distance of bacteria varied from 10-11mm. (Table1)

Antifungal activity of 5 different extracts of ascidians shows that maximum Inhibition zone of 17mm noticed in *Aspergillus niger* at ethanol extract and minimum Inhibition zone of 7mm noticed in chloroform and methanol extract of *Rhizopus stolonifer* (Table 1).

The present findings of Neda et al., 2014, antifungal activity of *Phallusia nigra* of Persian Gulf shows that maximum inhibition zone of 25mm observed in *Aspergillus niger* when the concentration increased from 5 to 20$\mu l$.

Table 2 Preliminary phytochemical screening of the various extracts of *Phallusia nigra*

| S. No | Test | Ethanol | Methanol | Ethyl acetate | Chloroform | Aqueous |
|---|---|---|---|---|---|---|
| 1. | Carbohydrate | - | - | - | + | - |
| 2. | Protein | + | + | - | - | - |
| 3. | Amino acid | + | - | - | - | - |
| 4. | Alkaloid | + | + | - | - | - |
| 5. | Flavonoid | + | + | - | - | - |
| 6. | Glycoside | - | - | + | + | - |
| 7. | Steroid | - | - | - | - | - |
| 8. | Saponin | + | - | - | - | - |
| 9. | Tannin | + | - | - | - | + |
| 10. | Phenol | - | - | - | + | - |

The phytochemical constituents like alkaloids, flavonoids, steroids, tannins, saponin, phenols, proteins, amino acids, carbohydrate, Glycosides were tested using 5 different extracts like ethanol, methanol, chloroform, aqueous and ethyl acetate. The results are presented in table 2. The ethanol and methanol showed the presence of alkaloid, and carbohydrate. The methanol extract showed the presence of alkaloids, flavonoids, proteins and amino acids. Aqueous extract showed the presence of phenols, carbohydrate and tannin

The knowledge of phytochemical screening is desirable, not only for the discovery of therapeutic agents but also because such information may be of value disclosing new sources of such economic materials, like tannins, oils, gums, precursor for the synthesis of complex chemical substances etc. Major phytochemical constituents like alkaloids, steroids, flavonoids, saponins are present in almost all extraction. Phenolic compounds saponins and flavonoids may be linked or suggested to be involved in antibacterial, antiviral and anti diarrheal activity as suggested by Majaw and Moirangham in plants. Investigations on the mode of action by Enzo in plants indicate that flavonoid increase colonic water, electrolytic re absorption and other chemicals act by inhaling intestinal mobility while some components have been show to inhibit particular enter pathogens. Alkaloids are reported to have cardiovascular effects by Juge et al. (2001). As ascidians are sedentary animals the same role may be suggested in these animals as defense mechanism.

Table 3 Quantitative estimation of phytochemicals

| Extract | Glycoside ug/ml | Flavanoid ug/ml | Saponin ug/ml | Protein ug/ml | Tannin ug/ml | Phenol ug/ml | Carbohydrate ug/ml | Amino Acid ug/ml |
|---|---|---|---|---|---|---|---|---|
| Methanol | - | 25 | - | 55 | - | - | - | - |
| Ethanol | - | 65 | 39 | 120 | 4 | - | - | 46 |
| Chloroform | 10 | - | - | - | - | 39 | 20 | - |
| Ethyl acetate | 13 | - | - | - | - | - | - | - |
| Aqueous | - | - | - | - | 5 | - | - | - |

Phytochemicals like glycoside, flavonoid, saponin, protein, phenol, carbohydrate and Amino acids are present in ascidian *Phallusia nigra*. Maximum production is protein 120$\mu g/ml$ in ethanol extract. Second maximum production is flavonoid 65$\mu$g/ml of ethanol extract and minimum production of Tannin 4$\mu$g/ml of ethanol extract only. (Table 3)

Phytochemicals play a vital role in the medicinal properties. Consumption of spices has been implicates in the prevention of many chronic diseases such as cardiovascular diseases, cancer and inflammation. (Panpati et al, 2013). Phenolic are good antioxidants and exhibit a wide range of pharmacological properties such as anticancer, anti-inflammatory and anti-diabetic effects (Hamazah et al., 2013). Flavanoids are group of more than 4000 polyphenol compounds that occur naturally in food of plant origin, possessing a number of beneficial effects to human health (Menaga et al., 2012). They are potent water soluble, super antioxidants and free radical scavengers that prevent anti-oxidative damage, and have anticancer, antiinflamatory, anti allergic, antiviral,vrasodilating properties and inhibition of platelet aggregation. Also it is known that saponins inhabitant efflux by blockage of the influx of concentration in the cells activating a Na+-ca+ antiporter in cardiac muscles. The increase in Ca2+ influx through this anti porter strengthens the contraction of cardiac muscles (Ergwim et al., 2011; Schneider et al., 2004)

Tannins may elicit antibacterial activities via cell membrane lysis, inhibition of protein synthesis, proteolytic enzymes and microbial adhesions. (Dulgar et al., 2002).Tannins is used to treat nonspecific diarrhoea and inflammation of the mouth).

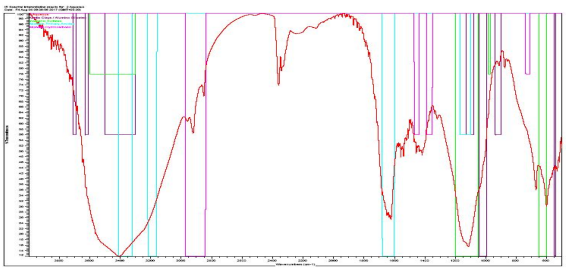

Fig. 1 FTIR Spectrum of the aqueous extract of *Phallusia nigra*

Table 4 Peak values of aqueous extract of *Phallusia nigra*

| Frequency ranges | Intensities | Assignment and remarks | Group or Functional Class |
|---|---|---|---|
| 3404 | Medium | O-H stretch | Phenols, alcohol |
| 2359.47 | Strong | O-H stretch | Carboxylic acids |

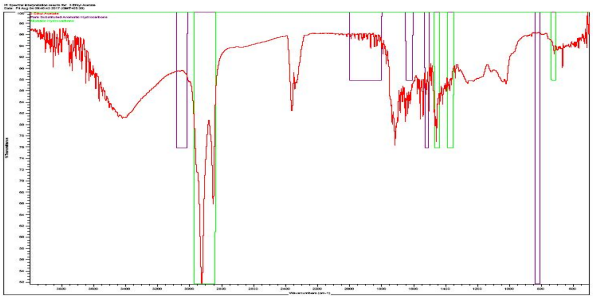

Fig. 2 FTIR Spectrum of Ethyl acetate extract of *Phallusia nigra*

Table 5 Peak values of FTIR Ethyl acetate extract of *Phallusia nigra*

| Frequency ranges | Intensities | Assignment and remarks | Group or Functional Class |
|---|---|---|---|
| 2924.15 | Strong | H-C-H Asymmetric stretch | Alkane |
| 2362.20 | Strong | Hydrogen bonded O-H stretch | Carboxylic acids |

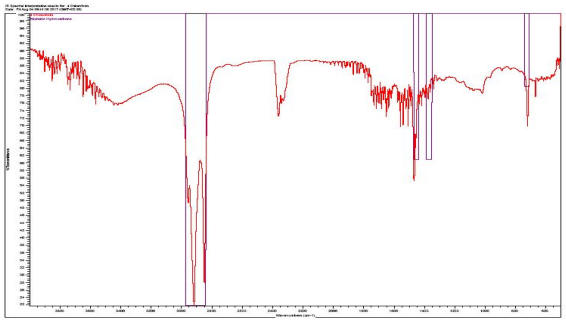

Fig.3 FTIR Spectrum of chloroform extract of *Phallusia nigra*

Table 6 Peak values of FTIR chloroform extract of *Phallusia nigra*

| Frequency ranges | Intensities | Assignment and remarks | Group or Functional Class |
|---|---|---|---|
| 2917.17 | Strong | H-C-H- Asymmetry and Symmetry | Alkane |
| 2819.76 | Strong | H-C-H- Asymmetry and Symmetry | Alkane |
| 2360.39 | Strong | Hydrogen bonded O-H stretch | Carboxylic acids |

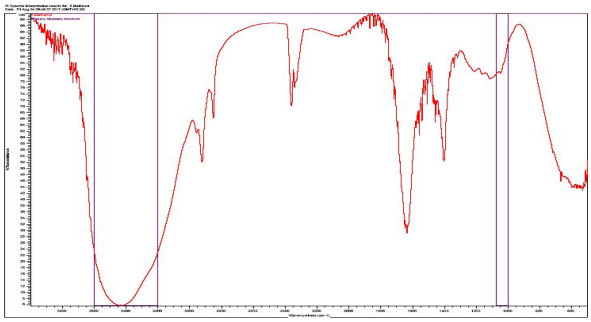

Fig. 4 FTIR Spectrum of Methanol extract of *Phallusia nigra*

Table 7 Peak values of the FTIR Methanol extract of *Phallusia nigra*

| Frequency ranges | Intensities | Assignment and remarks | Group or Functional Class |
|---|---|---|---|
| 3444.15 | Strong | N-H stretch | Amines |
| 2359.72 | Strong | Hydrogen bonded O-H stretch | Carboxylic acids |
| 1716.80 | Medium | C=O stretch | Ketone |
| 1404.24 | Strong | H-C-H Bond | Alkane |

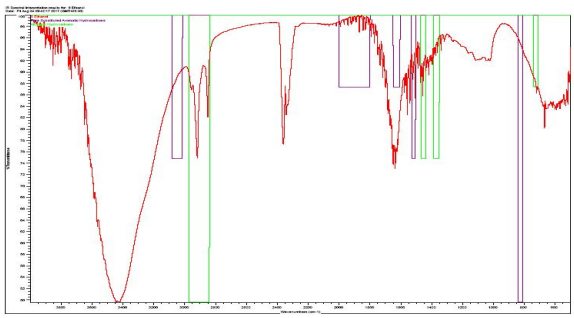

Fig. 5 FTIR Spectrum of Ethanol extract of *Phallusia nigra*

Table 8 Peak values of the FTIR Ethanol extract of *Phallusia nigra*

| Frequency ranges | Intensities | Assignment and remarks | Group or Functional Class |
|---|---|---|---|
| 3445.11 | Strong | N-H stretch | Amines |
| 2918.11 | Strong | C-C-H Asymmetric stretch | Benzene Aromatic rings |
| 2359.72 | Strong | Hydrogen bonded O-H Stretch | Carboxylic acids |
| 1500.4 | Medium | N= O stretch | Nitro group |

Aqueous extract of *Phallusia nigra* was analysed by FTIR (Fig. 1). It has phenol and alcobol with OH stretch (3404) carboxylic acids OH (2359.47) (Table 4). *P.nigra* ethylacetate extract analysed by FTIR (fig2) It has the compound Alkane H-C-H stretch (2924.15) carbosylic acids with O-H stretch (Table-5) P.nigra chloroform extract were analysed by FTIR (Fig. 3) it has the alkane group H-C-H Asymmetry and symmetry (2917.17), (2819.76) and carboxylic acids –O-H stretch (2360.39) (Table 6). P.nigra methanol extract was analysed by FTIR (Fig. 4) it has the amines N-H stretch (3444.15) carboxylic acids hydrogen bonded O-H stretch (2359.72) ketone with c=O stretch (1716.80) and alkane H-C-H bond (1404.24) (Table7). P.nigra of ethanol extract was analysed by FTIR (Fig. 5) it has the benzene Aromatic rings with C-C-H stretch (2918.11) carboxylic acids O-H stretch (2359.72) and nitro group with N=O stretch (1500.4) (Table8).

However crude extracts of ascidians *Phallusia nigra* with many compounds and their active portion may be very low. FTIR major peaks showed that it have phenol, methane, ketone and carboxylic acid group as a major groups in the ascidians. Phenolic compounds exhibited good antioxidant andantimicrobial activities.

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