International Journal of Pure and Applied Zoology
Volume 2, Issue 3, pp: 218-223, 2014

ISSN (Print) : 2320-9577
ISSN (Online): 2320-9585
http://www.ijpaz.com

Research Article

ISOLATION AND CHARACTERIZATION OF BIOACTIVE COMPOUNDS AND ANTIBACTERIAL ACTIVITY OF MARINE GASTROPOD *PHALIUM GLAUCUM* (L)

R.D.Thilaga*, S.Vimala and P.Subavathy
St.Mary's College (Autonomous) Thoothukudi-628 001, Tamil Nadu, India

Article History: Received 25th November 2013; Accepted 9th July 2014; Published 19th July 2014

ABSTRACT

The whole body extract of *Phalium glaucum* with different column chromatographic fractions were assayed for antibacterialactivity using disc diffusion technique against six human pathogenic bacteria.Among the five column purified extracts F1 and F4 fractions exhibited wide spectrum activities. Highest activity was recorded against S.typhi (12 mm) and S.flexneri(9 mm) of F4 and F1 fractions respectively. Through GC-MS analysis 10 compounds were identified and charecterised. Of the 10 compounds hexanal-2-methyl,1,2-benzene dicarboxylic acid, diisooctyl ester,2,2-dimethyl propionic acid, hexadecyl ester and pseudoephedrine might be responsible for antimicrobial activity.

Keywords: *Phalium glaucum*, GC-MS analysis, Bioactive compounds, Antibacterial activity.

INTRODUCTION

Ocean offers a large biodiversity of fauna and flora which is estimated to be over 5,00,000 species and more than double that of land (Anand *et at.*, 1997). The marine environment is a huge source for yet to be discovered natural products. Apart from human medicines, the research on marine natural products in the last three decades has also brought the discoveries of many chemically and biologically interesting molecules that have become indispensable tools in biochemical research and has played significant roles in the recent advancement of life sciences (Umayaparavathi *et al.*, 2012).

Molluscs are said to be pharmacologically significant out let. There are more than thousands of bioactive compounds discovered in molluscs. They are pedtide, depsipedtide, sterols, sesquiterpenes, terpenes, polypropionate, nitrogenous compounds, macrolides, prostaglandins miscellaneous compounds and alkaloids (Blunt *et al.*, 2006). Perusal of literature revealed that a large number of works have been carried out in other groups of organisms but only a few studies were made in molluscs (Anand and Patterson Edward 2001; Mary Elizabeth *et al.*, 2003; Thilaga, 2005; Annamalai *et al.*, 2007; Chandran *et al.*, 2009; Maripandiet *et al.*, 2010; Periasamy *et al.*, 2011; Ramasamy Mariappan and Balasubramanian, 2012) The availability of molluscs is too high and their utilization is extremely low compared to other marine organisms. Hence the present investigation was undertaken to explore the antibacterial activity and also isolate and characterize the bioactive compounds from the whole body tissue extract of *Phalium glaucum.*

*Corresponding author e-mail: rdthilaga12@gmail.com

MATERIAL AND METHODS

The gastropod *P. glaucum* was collected from Gulf of Mannar coastal region of Thoothukudi by trawl nets used for capturing the crab, brought to the laboratory, cleaned and washed with fresh sea water to remove all impurities. The shells were removed and the tissues were then dried in hot air over at 56° C for 48 hours. The dried tissue was immersed in 100% AR grade methanol for 10 days at room temperature. After filtration with Whatman No. 1 paper, the methanol extract was reduced by vacuum evaporation. The extract residue was resuspended in 20 ml of 100% A.R. grade methanol. The methanol soluble extracts were dried and solidified in deionized water. Different concentrations of extracts were prepared and stored at 0° C for further studies.

The crude extract was fractionated by silica gel column chromatography with five solvent systems viz. Hexane: chloroform (1:1) (F1), chloroform (F2), benzene (F3) methanol: benzene (1:1) (F4) and methylene chloride(F5). A known amount of extracts were taken and their organic solvents were removed by vacuum evaporation. Solids were dissolved in deionized water and concentration series of 1mg/ 1ml, 10 mg/ml and 100 mg/ml were prepared and used.

Antibacterial assay

Six species of pathogenic bacteria namely *Esherichia coli, Klebsiellapneumoniae, Proteus vulgaris, Salmonella typhi, Shigella flexneri* and *Staphylococcus aureus* were obtained from Microbiology Department, Sri Paramakalyani College, Alwarkurichi. Nutrient both medium was prepared and sterilized in an autoclave at 151b pressure.These bacterial species were inoculated in the nutrient broth at 37° C for 24 h. Then the pathogens were swabbed on the surface of the Nutrient agar (Himedia) plates and discs (Whatman No. 1 fitter paper 6 mm diameter) impregnated with different concentrations (1 mg/ml, 10 mg/ml and 100 mg/ml) of the extract of *P. glaucum* were placed on the surface along with control discs. The plates were incubated at 37° C for 24 h and the antibacterial activity was measured based on the inhibition zone around the impregnated discs. The same procedure was adopted for crude as well as all the fractions (F1 to F5). The more potent fraction was characterized to know the various compounds through GC- MS study.

GC - MS analysis

GC-MS analysis was carried out on a Gc Clarus 500 Perkin Elmer system composing a Aoc 20i auto sampler and gas chromatography interfaced to a mass spectrophotometer (GC- MS) instrument employing the following conditions such as column elite-5MS fused silica capillary column Elite – 1 (30 x 0.25 mm I D x 1 EM dg, composed of Dimethyl polysiloxane) operating in electron impact mode at 70 ev; Helium (99.999%) was used as a carrier gas at a constant flow of 1 ml/min and an injection volume of 0.5EI (split ratio of 10:1) injection temperature 250° C ; and ion source temperature 280° C. The oven temperature was programmed from 110° C (isothermal for 2 min), with an increase of 10° C /min to 200° C, then 5° C/min to 280° C interval of 0.5 S and fragments from 40 to 500 Da.

Interpretation on mass spectrum was conducted using the data base of NIST ver 2.1, WILEY8 and FAME having more than 62,000 patterns. The unknown components found in the body tissues of *P. glaucum* were matched with the NIST, WILEY8 and FAME, the M S library and predicted from Dukes Ethno Botanical Database.

RESULTS

The crude methanol extract of *P. glaucum* showed highest activity against *Salmonella typhi* (9 mm) followed by *S. flexheri* (6 mm)

and *S. aureus* (5 mm). The extract showed very weak activity against *K. pneumoniae* (1 mm) (Figure 1). In fraction 1(F1), highest activity was recored against *S. flexneri* (9 mm) and *S. typhi* (7 mm) and the lowest activity was noticed against *E. coli* (3 mm) (Figure 2).

In F2 fraction maximum activity was recorded against *Salmonella typhi* (6 mm) and minimum activity against *K. pneumoniae* and *S. aureus* (2 mm) (F Figure ig 2). In fraction 3 (F3) maximum activity was recorded against *Salmonella typhi*(6 mm) and minimum activity against *K. pneumoniae* and *E coli* (2 mm) (Figure 2). In fraction 5 (F5) the highest activity was observed against *S. flexheri* (5 mm) and lowest activity was noticed against *E. coli* and *K. pneumonia*. Among the five extracts tested fraction 4 (F4) showed wide speetrum activity by developing 12 mm inhibition zone against *Salmonella typhi*, 10 mm against *S. flexneri* and the minimum zone was observed against *E. coli* (5 mm) (Figure 2). In the present study, among the concentrations tested 100 mg/ml concentration exhibited maximum activity and 1mg/ml concentration showed minimum activity. GC-MS study revealed the presence of four antibacterial compounds in the body tissue extract of *P. glaucum* (Table 1).

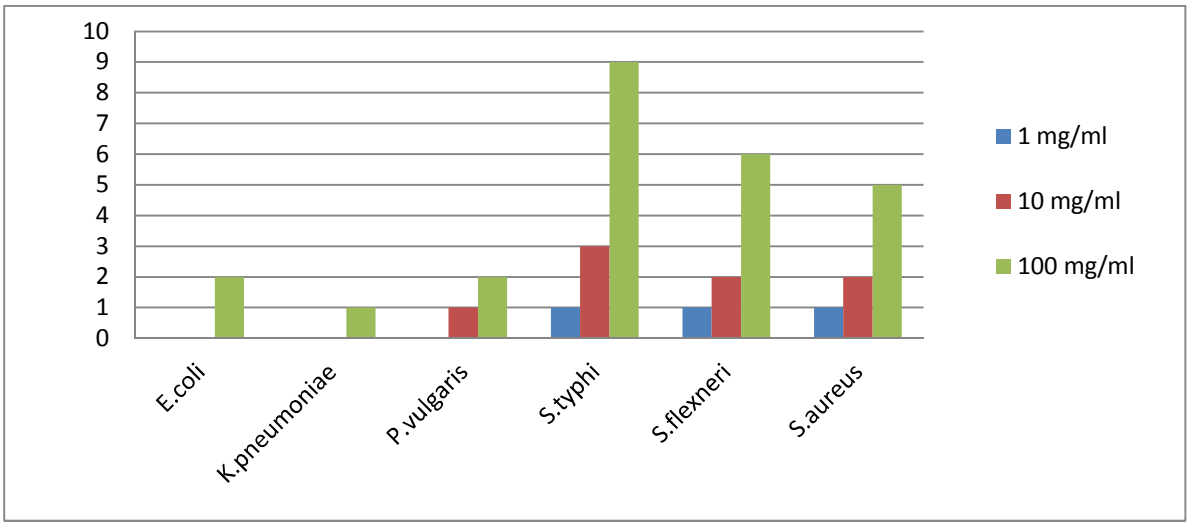
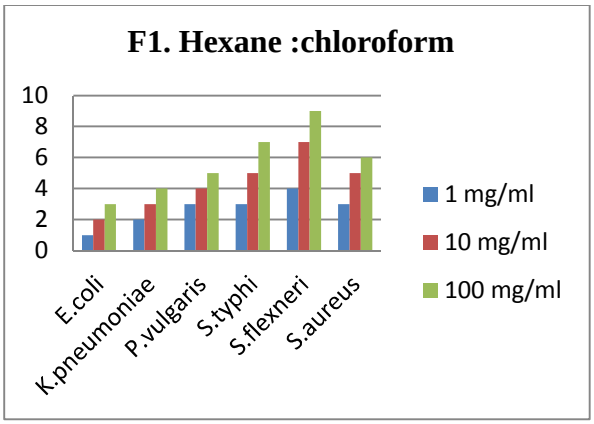
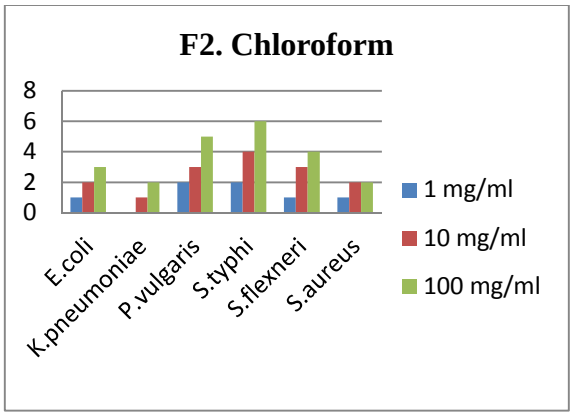


Figure 1. Antibacterial activity of crude methanol extracts of *P. glaucum.*





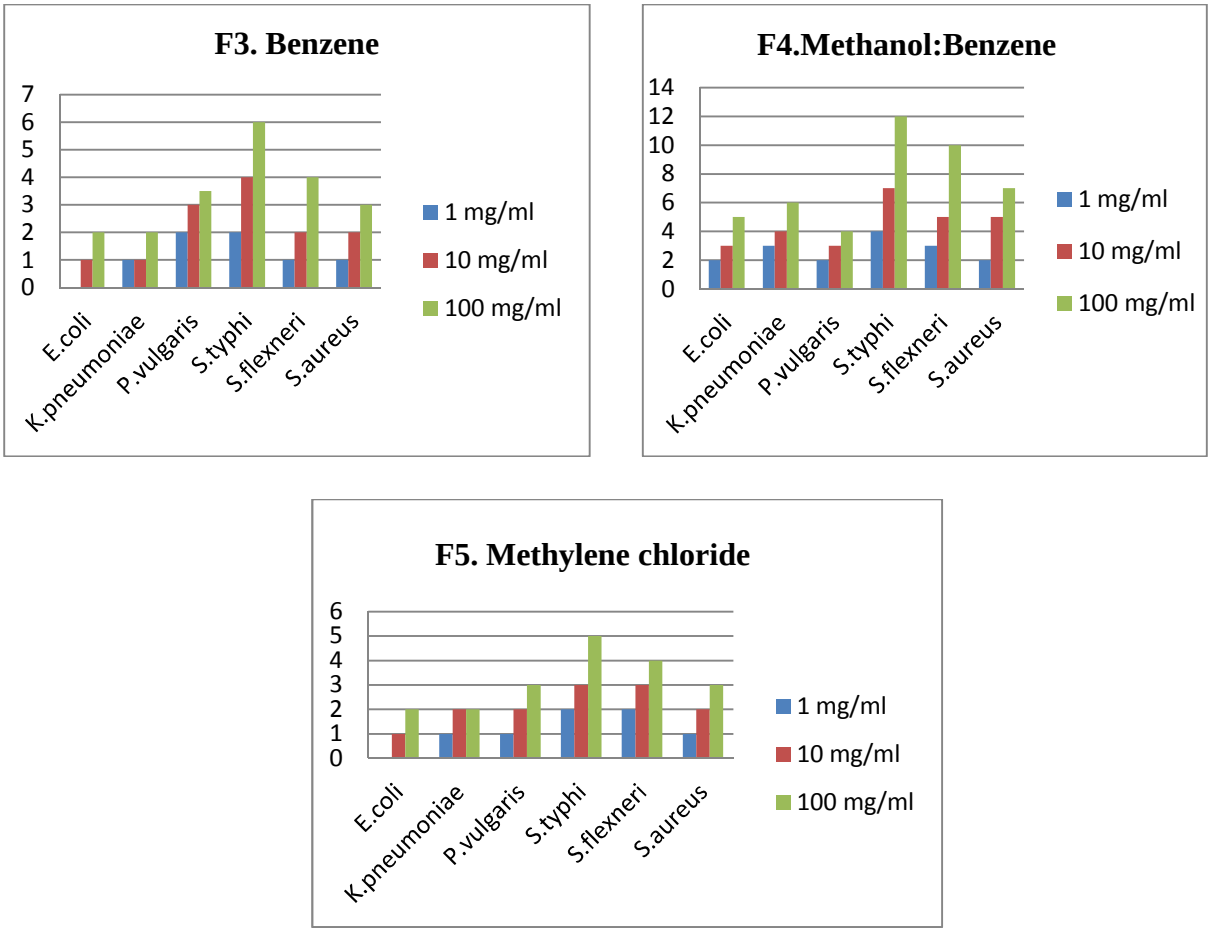


Figure 2. Antibacterial activity of column fractionated extract of *P.glaucum* against pathogen.

Table 1. Activity of Components identified in the column fractions of Phalium glaucum by GC-MS.

No.	RT	Name of the compound	Molecular formula	MW	Peak Area %	Nature of compound	**Activity
1	14.93	Diallylmethylsilane	$C_7H_{14}Si$	126	0.25	Silica compound	No activity reported
2	16.81	Hexanal, 2-methyl-	$C_7H_{14}O$	114	0.31	Aldehyde	Antimicrobial Anti-inflammatory
3	18.36	Hexanoic acid, octadecyl ester	$C_{24}H_{48}O_2$	368	0.25	Fatty acid ester	No activity reported
4	20.28	1,2-Benzenedicarboxylic acid, diisooctyl ester	$C_{24}H_{38}O_4$	390	0.58	Plasticizer compound	Antimicrobial Antifouling
5	20.81	2,2-Dimethylpropionic acid, hexadecyl ester	$C_{21}H_{42}O_2$	326	0.39	Propionic acid ester	Antimicrobial
6	21.28	Pseudoephedrine, (+)-	$C_{10}H_{15}NO$	165	0.58	Alkaloid	Antimicrobial Antiinflammatory
7	24.06	6,11-Dimethyl-2,6,10-dodecatrien-1-ol	$C_{14}H_{24}O$	208	0.26	Alkene compound	No activity reported
8	27.53	6,9,12-Octadecatrienoic acid, phenylmethyl ester, (Z,Z,Z)-	$C_{25}H_{36}O_2$	368	1.29	Linoleneic acid ester	Cancer preventive Anti-inflammatory Hepatoprotective Nematicide Antihistamic Hypocholesterolemic Antiarthritic Antiacne 5-Alpha reductase inhibitor Insectifuge
9	28.14	Cholesterol	$C_{27}H_{46}O$	386	88.45	Steroid	Antioxidant
10	28.69	Spiro[androst-5-ene-17,1'-cyclobutan]-2'-one, 3-hydroxy-, (3á,17á)-	$C_{22}H_{32}O_2$	328	4.29	Ketone compound	No activity reported

DISCUSSION

In the present investigation a prominent antibacterial activity has been observed against some bacterial strains. Fraction four of *P. glaucum* showed wide spectrum activity. The maximum zone of inhibition was recorded against *S. typhi* (12 mm) strain and minimum zone of inhibition was observed against *E. coli* (5 mm). The study of coral associated gastropod, *Trochus tentorium* against the human pathogens had shown similar result (Anbuselvi *et al.,* 2009).

In this investigation, *P. glaucum* has shown promising results which fuels the need to isolate their bioactive compounds and to test them against a wider array of pathogens. The considerable activity of *P. glaucum* against *S. typhi* and weak activity against *E.coli* is contrary to the observation made by Anand *et al.* (1997). In their study, the methanolic extract of *H.pugilinus* inhibited *E. Coli, N. subtilis* and *K. pneumoniae* significantly while no activity was observed against *V. cholera* and *S. pyogenes.* This could be due to the variation in the geographic location, food availability and climatic conditions of the organisms tested in the study.

In the present study distinct antibacterial activity was observed against almost all pathogenic bacteria. F_4 showed highest activity against *S. typhi* (12 mm) and *S. flexneri* (10 mm) and the F1 fraction. against *S. flexneri* (9 mm) and *S. typhi* (7 mm). Kanagasabapathy *et al*. (2011) observed the antibacterial activity of melomelo. Dhinakaran *et al.* (2011) observed the anti pathogenic activity of marine gastropod *H.pugilinus* against few pathogens and the ethanol and methanol extracts showed significant activity against *Proteus mirabilis* and *E. coli.* Thilaga (2005) and Jayaprabha (2010) studied the antibacterial activities in the ethanol extracts of gastropods *Babylonia spimata and Turbo brunneus* and observed highest activity against *E. coli K. preumoniae, P. vulgaris and S. typhi.* Sugesh *et al.* (2013) noticed highest antibacterial activity with ethanol and methonol extract of *H. puglinus* against human pathogens. All the above findings agree with with the observations of the present study.

In the present study GC-MS analysis and antibacterial activity of *P. glaucum* showed the presents of bioactive compounds responsible for antibacterial activities. The presence of antibacterial compoundsin the oyster *Pteria chinersis* and bivalve *Perna viridis* have been reported using the various solvent extracts (Chellaran *et at.,* 2004; Li *et at.* (2009); Ding *et at.,* 2011). In the present study the magnitude of inhibition of column fractionated extract of *P. glaucum* possibly reveals the presence of antimicrobial compounds. Hexanal–2 methyl, 1,2-Benzena dicarboxylic acid, diisooctyl ester, 2,2–Dimethyl propionic acid, hexadecyl ester, and pseudoephedrine identified from GC-MS analysis might be responsible for antimicrobial activity. As the molluscan resources are rich and varied in Indian coasts, there exist a great potential for the extraction of bioactive compounds of medicinal importance at a lower cost.

CONCLUSION

There is an urgent need for the discovery of new and novel antibacterial drugs to effectively combat not only the drug resistance but also the disease producers. The present study concludes that the new models and mechanisms of action of body tissue extract of *P. glaucum* will bring new solutions for tackling some of the major public health problems.

CONFLICTS OF INTEREST

The authors declare that there are no conflicts of interest associated with this article.

ACKNOWLEDGEMENT

The authors are grateful to UGC, Govt. of India, New Delhi for providing financial assistance and to Dr. Sr. S. Decla, Principal, St. Mary's College (Autonomous), Thoothukudi for providing the necessary laboratory facility.

REFERENCES

Anand, T.P. and Patterson Edward, J.K., 2001. Screening for antibacterial activity in the opercula of gastropods. *Phuket Mar. Biol. Centre Spl. Pub.,* 25: 215-217.

Anand, T.P., Rajaganapathy, J. and Edward, J.K.P., 1997. Antibacterial activity of marine mollusks from Portonovo region. *Ind. J. Mar. Sci.,* 26: 206-208.

Anbuselvi, S., Chellaram, C., Jones, S., Jayanthi, A. and Edward, J.K.P., 2009. Bioactive potential of coral associated gastropod, *Trochus tentorium* of gulf of mannar, Southeastern India. *J. Med. Sci.*, 9:240-244.

Annamalai, N. Anburaj, R., Jayalakshmi, S., Thavasi, R., 2007. Antibacterial activities of green mussel and edible oyster *Crassostrea madrasensis*. *Res. J. Microbiol.,* 2(12): 978-982.

Blunt, J.W., Copp, B.R., Munro, M.H.G. Nothcote, P.T. and Prinsep, M.R., 2006. Natural products from Marine organism and their associated microbes. Nat. Prod. Rep., 23:26-78.

Chandran, B., Ramesh Kumar, G, Ravichandran, S., 2009. Antimicrobial activity from the gill extraction of *Pernaviridis* (Linnaeus 1758). *Global J. Biotech Biochem.,* 4(2): 88-92.

Chellaram, C., Gnanambal, K.M. and Edward, J.K.P., 2004. Antibacterial activity of the winged oyster, *Pteria chinensis* (Pterioidae: Pteridae). *Indian J. Mar. Sci.,* 33: 369-372.

Dhinakaran, A., Sekar. V, Sethubathi, G.V., Suriya, J., 2011. Antipathogenic activity of marine Gastropoda (*Hemifusus pugilinus*) from Pazhayar, South east coast of India. *Int. J. Environ. Sci.,* 2(2): 524-530.

Ding, G.F., Huang, F.F., Yang. Z.S., Yu. D. and Yang, Y.F., 2011. Anticancer activity of an oligopeptide isolated from hydrolysates of *Sepia* ink. *Chin. J. Nat. Med.,* 9: 151-155.

Jayaprabha, D., 2012. Studies on some biological aspects of *Turbo brunneus* (L.1758) along the Gulf of Mannar region of Thoothukudi. Ph.D. Thesis, Manonmaniam Sundaranar University, Tamil Nadu, India.

Kanagasabapathy S., Ravichandran, S. and Kumaresan, M., 2011. Preliminary studies for anew antibiotic from marine mollusk *Melo melo* (Lightfoot,1786). *Asian Pacific J. Trop. Med*., 4(4): 310-314.

Li, C., Song, I., and Zhao, J., 2009. A rewiew of advances in research on marine molluscan antimicrobial peptides and their potential application in aquaculture. *Molluscan Res*., 29: 17-26.

Maripandi, A., Prakshali, L. and Salamah, A.I., 2010. HPTLC separation of antibacterial compounds from *Perna viridis* and *Sanguino lentus* and its activity tested against common bacterial pathogens. *Advanced Biotech*., 9(9): 24-28.

Mary Elezabeth, K.G., Chellaram, C. and Jamila, P., 2003. Antimicrobial activity of reef associated gastropod, *Trochus radiatus.* National Seminar on Ecosystem, Remediation. p. 68.

Periasamy, N., Srinivasan, M. and Balakrishnan, S., 2011. Antibacterial 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.