Comparative evaluation of antibacterial activity of foot muscle extracts from genus Physa and genus Ceciloides (Mollusca: Gastropoda)

Javaria Altaf[1], Muhammad Hidayat Rasool[2], Saira Akhtar[1], Maleeha Manzoor[1], Tahira Younas[1], Bakhtawer Ansari[1] Gulraiz Ahmad[3], Farhat Jabeen[1], Muhammad Ali[1] and Rizwan Munir[4]

[1]Department of Zoology, Government College University, Faisalabad, Pakistan
[2]Department of Microbiology, Government College University, Faisalabad, Pakistan
[3]Department of Biochemistry, Government College University, Faisalabad, Pakistan
[4]Department of Statistics, Government College University, Faisalabad, Pakistan

Abstract: The aim of this study is to explore the presence of antimicrobial bioactive agents in the foot muscle extracts of snails belonging to genus Physa and Ceciloides. Antibacterial activity of foot extracts belonging to species named as *P. fontinalis*, *P. gyrina*, *P. acuta*, *C. acicula*, *C. eulima*, *C. petitiana*, was checked and compared against three bacterial strains i.e. *E.coli*, *P. auroginosa*, *S. aureus* by using disc diffusion method. The results were highly significant with maximum zone of inhibition of 20.10 mm in the *P. fontinalis* acetone extract and the least was 12.97 mm of *C. eulima* diethyl ether extract. The microdilution method was employed to observe MIC to evaluate antimicrobial resistance pattern of snails foot muscle extract against three mentioned strains. MIC of foot extracts was ranging from 0.03ug/ml-5 ug/ml for six species. TLC was carried out for profiling of extracts with positive results. Foot extracts from species of both genera eluted in different fractions of compounds with a good resolution in 100% n-hexane and ethyl acetate each. The plates developed in solvent system showed purple and yellow spots indicating the presence proteins and organic compounds showing it a promising canditadate for the therapeutic purposes.

Keywords: Physa, Ceciloides, foot extracts, MIC, TLC, disc diffusion technique.

INTRODUCTION

Bacterial resistance develops as a survival mechanism, due to genetic changes, which inturn results in the modification of the enzymes that break the linkage of the antibiotics with the target proteins, as a result of which there is an increasing trend of decreasing effectiveness of the antibiotics in the treatment of the common infections (Laxminarayan *et al.*, 2013; Sanae *et al.*, 2003; Abiona *et al*., 2015). Due to this there is a vital interest in scientist to discover novel antimicrobial compounds which at one hand poses fewer environmental and toxicological risks and with minimum chance of resistance development by the pathogens. There is a huge resource of the bioactive natural compounds in the marine environment which can be further isolated and characterized for the treatment of the disease (Periyasamy, 2012). Approximately 7000 bioactive compounds have been derived from various marine invertebrate phyla (Chellaram *et al*., 2004) including various classes of Phylum Mollusca for antiseptic properties. This bioactivity may be due to the reason environmental pressures on the animals, with sedentary mode of life leading to the survival of those organisms who can produce bioactive metabolites to combat these ecological pressures successfully (Proksch, 2002). In molluscs the pharmacological properties with biomedical importance have been found quite pronounced in molluscs, for various therapeutic applications, (Dolashka-Angelova *et al*., 2008) and a number of them have been discovered in recent years. Snails belong to the class Gastropoda and land snails are one of the most numerous with almost 35,000 described species of the world. In the gastropods a range of antimicrobial peptides have been found (Bergsson *et al.*, 2005). In most of the Indian medicines, the gastropod operculum is being used as a major ingredient to fight off various diseases (Periyasamy, 2012). The hemolymph and the whole body homogenates of the snails have been reported to contain a variety of bioactive compounds such as peptides, glycopeptides and glycans (De Smet *et al*., 2011; Periyasamy, 2012), mucin, epiphragm in molluscan groups (Etim *et al*., 2016) which have been found to have strong inhibitory action against viruses bacteria and protozoan mixed cultures (Li *et al.*, 2011).These are valuable as they are synthesized from very small molecules ranging from from 5 to 15 KDa and being part of the animals' natural defence system they also reach to the point of infection very quickly (Periyasamy, 2012; Soderhal *et al.*, 2011). These are not only gaining attention due to antimicrobial properties but are also strongly effective being anticancerous and antiviral, however, further studies on them is the need of time (Dolashka *et al*., 2011). Mytimacins, myticins, mytilins anddefensins are the examples which have been characterized from molluscs (Gerdol *et al.*, 2012).

**Corresponding author:* e-mail: javariaaltafuaar@yahoo.com

Most of the researches that have been conducted are on the biodiversity of the invertebrates and snails (Ali, 2005; Rehman, 2010) and snails have been given the status of a pest threatening the agricultural yield and being themselves targeted by the chemical pesticides. Considering the rich diversity and high commercial value of marine molluscs, it has to be noted that only negligible number of species has been investigated so far with respect to antimicrobial agents with a high potential of anticancerous activities (Suarez-Jimenez, *et al*., 2012). Previous studies show that three snails species i.e *P. fontinalis*, *Z. insularis* and *C. acicula* were found completely absent from most of the villages except a few (Altaf, 2017). The villages where these specimens have been found are considered to be as the areas from where we can find the resistant members of the population. These are the important samples for the isolation of antibacterial bioactive agents. So, this has been hypothesized that they may have some important natural antibacterial agents/substances in their bodies which support their lives in such environment. Terrestrial gastropods are generally considered as pests and are sprayed with strong pesticides; however, they can be harvested for their body extracts for further discoveries in the best interest human, i.e. in the form of medicines and saving the environment from pesticides.

MATERIALS AND METHODS

Study area

The third largest and populous city of Pakistan, Faisalabad, is situated in the central Punjab covered an area of 122 km^2 at spherical coordinates of $31^0 25^/$ N and $73^0 09^/$ E located at an altitude of 300 m above mean sea level (Kahlown, 2006). This city was selected for species collection as a result of the reports of declining population species belonging to Genus Physa i.e. *P. fontinalis, P. gyrina, P. acuminata* and Genus Ceciloides *C. acicula, C. eulima, C. petitiana* (Altaf, 2017) for the screening of antimicrobial activity.

Sampling and Identification

The sampling was carried out from agricultural fields of Faisalabad, with the help of the local farmers at the dawn and dusk from October 2015 to November 2016. On site identification was carried out upto genus however, later the snail samples were brought to the lab and were identified upto species following (Subba Rao, 2003).

Extraction of antimicrobial compounds

The shells of the suffocated snails were carefully removed. The foot muscles extracts were prepared in Ethanol, diethyl ether and acetone, separately for the extraction of their bioactive compounds following Chellaram *et al* (2004). The gummy mass obtained after extraction was stored at 4°C to avoid contamination and for further analysis.

Antibacterial assay

Standard disc diffusion method was used to evaluate antibacterial activity of the prepared extracts against *S. aureus*, *E. coli, P. auroginosa* following Periyasamy (2012). A clear area with no growth was observed around that particular disk (Periyasamy, 2012). It means the test isolate were susceptible (Schwalbe *et al.*, 2007). This is qualitative testing method, MIC measurement cannot be determined. This test simply classifies the isolate as susceptible, intermediate or resistant. (Pelczar *et al*., 1998)

Minimum inhibitory concentration (MIC)

The MIC (Minimal Inhibitory Concentration) of a bacterium to a certain antimicrobial agent can be determined and it gives the finest quantitative approximation for susceptibility of antimicrobial agent required to inhibit growth of the bacteria. The experiment was optimized following Andrews (2001).

Thin layer chromatography

Thin layer chromatography was done to confirm the presence of compound which inhibit the growth of bacteria and show antibacterial activity. Ethyl acetate 100% and n-hexane 100% was pour in beakers. After that each TLC plates was placed in the beaker and observe the plate as solvent move upward. Plates were taken out from the beaker and let them dry. Then each TLC plate was seen in UV lamp to check the presence of compound. In UV light they gave purple and yellow spots which indicate the presence of compounds.

STATISTICAL ANALYSIS

The data was subject to statistical analysis for the Analysis of Variance using Minitab 17.

RESULTS

Disc diffusion method

Antimicrobial activity of snail species in different extracts against pathogens

The highly active species foot extracts among six species, *P. acuminata* showed maximum zone of inhibition against *P. auroginosa* was (19.3 mm). The best antimicrobial activity of ethanol extract of snail against *E. coli* was observed as (19.03mm) zone of inhibitions followed by *S. aureus* (17.77mm) in *P. gyrina*. Next to that, in Cecilioides best activity was found against *E. coli* sp. (16.13mm) in *C. petitiana* and the least activity was observed in *C. petitiana* (13.17 mm) against *P. auroginosa* (table 1, figs. 1-3)**.**

The highest antimicrobial activity of foot extract in ethanol, found against *P. auroginosa* was by *P. fontinalis* (20.10 mm) followed by foot extract of *C. petitiana* with a zone of inhibition of19.27 mm, 18.73mm in *C. acicula* and 18.1mm in *P. acuminata* and 17.17mm in *P. gyrina*

with the least in *C. eulima* (15.03mm). The highest zone of inhibitions for *E. coli* (19.10 mm) was by the foot extracts of *P. acuminata* followed by *P. fontinalis* i.e., 17.2 mm and the least was found in *C. petitiana* (14.5mm). The values of zone of inhibition ranges from 16.83-16.9 in all the other species. The best activity against *P. auroginosa* was seen in *P. fontinalis* i.e. the zone of inhibition of 18.70 mm followed by *C. petitiana* (17.23mm). The value of the other three species i.e., *C.acicula*, *P. acuminata* and *P. gyrina* was 14.77mm, 15.20mm, 15.53mm. The least activity was observed in *C. eulimia* against *P. auroginosa* (14.1mm) 19.0 mm).

The highest antimicrobial activity of di-ethyl ether foot extract of snail was observed against *S. aureus* as (19.70 mm) zone of inhibition in *P. gyrina* and was followed by *P. fontinalis* (19.20 mm). This trend was followed in foot extracts of *P. fontinalis* against *E.coli* (18.13mm) with the same zone of inhibition in *C. acicula* foot extract against *S. aureus* (18.13 mm). 18.07 mm zone of inhibition by the same extract against *E.coli* followed by *C. acicula* (17.07mm) and the least activity was observed in *P. auroginosa* (12.97 mm). The range of zone of inhibition fall between14.1mm- 16.1mm.

The highest antimicrobial activity of foot extract in acetone found against *S. aureus* was by *P. fontinalis* (20.10 mm) followed by foot extract of *C. petitiana* with a zone of inhibition of 19.27 mm, 18.73mm in *C. acicula* and 18.1mm in *P. acuminata* and 17.17mm in *P. gyrina* with the least in *C. eulima* (15.03mm). The highest zone of inhibitions for *E.coli* (19.10 mm) was by the foot extracts of *P. acuminata* followed by *P. fontinalis* i.e.17.2 mm and the least was found in *C. petitiana* (14.5mm). The values of zone of inhibition ranges from 16.83-16.9 in all the other species. The best activity against *P. auroginosa* was seen in *P. fontinalis* i.e. the zone of inhibition of 18.70 mm followed by *C. petitiana* (17.23mm). The value of the other three species i.e. *C. acicula*, *P. acuminata* and *P. gyrina* was 14.77mm, 15.20mm, 15.53mm. The least activity was observed in *C. eulimia* against *P. auroginosa* (14.1mm) 19.0 mm) (table 1, figs. 1-3).

Minimum inhibitory concentration (MIC) determination by broth dilution method

MIC values range from 0.01ug/ml to 5 µg/ml in ethanol foot extract of snail species against three bacterial strains named *E.coli*, *P. auroginosa* and *S. aureus*. Minimum inhibitory concentration was 0.01µg /ml observed in the foot extract of *P. gyrina* followed by *P. fontinalis* and *P. acuminata* i.e. 0.03µg /ml and 0.07µg /ml respectively against all of three pathogens. In case of the members of the genus ceciloides the MIC was observed as 0.01, 0.07,0.6 against *S. aureus.* However the values against the *E. coli* and *P. auroginosa* are 1.25µg/ml, 2.5µg/ml and 5 µg/ml (table 2, fig. 4).

The ethanol foot extracts of *P. gyrina*, *P. acuminata* and *P. fontinalis* are found to be effective at low concentrations against all the bacterial strains followed by *C. petitiana* and *C. acicula*.

The C. eulima is least effective except against S. aureus. MIC values range from 0.07ug/ml to 5 µg/ml in acetone foot extract of snail species against three bacterial strains named *E.coli*, *P. auroginosa* and *S. aureus*. Minimum inhibitory concentration was 0.07 ug/ml observed in the foot extract of *P. fontinalis* against *S. aureus* followed by *C. acicula* (0.3 ug/ml) and *P. gyrina* (0.6 µg /ml) respectively. The MIC observed in *P. acuminata* and *C. eulima* is 1.25ug/ml followed by *C. petitiana* i.e. 2.5 µg/ml.

The MIC observed against *E. coli* was found 0.09µg /ml in *P. fontinalis* followed by 0.3µg /ml. The MIC observed in the *P. gyrina* and *C. eulima* extract was 2.25 µg/ml. The maximum value of MIC was observed in *C. petitiana* (5 µg /ml).

The MIC observed against *P. auroginosa* was 0.6 µg/ml followed by *P. gyrina*. The maximum value of MIC was observed against *P. auroginosa* in foot extracts of *C. eulima* except against *S. aueus.* The organisms were inhibited in the range between 5µl to 0.009µl by the diethyl ether extract of snail. MIC 0.09 µl was recorded in *P. fontinalis* against *E.coli* followed by *P. gyrina* 0.09 ul against *S.aureus* MIC values range from 0.09µg/ml to 5 µg/ml in diethyl ether foot extract of snail species against three bacterial strains named *E.coli*, *P. auroginosa* and *S. aureus*. Minimum inhibitory concentration was 0.09 µg/ml observed in the foot extract of *P. fontinalis* against *E.coli* followed by *P. acuminata* (0.3µg/ml) and *C. petitiana* (1.25µg/ml) respectively. The MIC observed in *P. gyrina* and *C. acicula* is 2.5µg/ml followed by C. *eulima* i.e. 5 µg/ml.

The MIC observed against *S. aureus* was found 0.09µg/ml in *P. gyrina* followed by *P. fontinalis* and *C. petitiana* 0.1µg/ml and *P. acuminata* 0.3µg/ml. The MIC observed in the *C. acicula* is1.25µg/ml followed by *C. eulima* extract was 2.5µg/ml.

The MIC observed against *P. auroginosa* was 0.6 µg/ml found in foot extracts of *P. acuminata* and *C. acicula* followed by *P. fontinalis* (1.25µg/ml). The minimum inhibitory concentration was 2.5µg/ml in *P. gyrina* and *C. petitiana*. The maximum value of MIC was observed against *P. auroginosa* in foot extracts of *C. eulima* except against *S. aueus.*

Analysis of Variance among snail species, bacterial strains and foot extracts, using Adjusted SS for Test

General Linear Model: Zone of inhibitions versus Snail species, strains, and Extracts

We use all six species of both genus Physa and Ceciloides in three extracts against three strains of bacteria in 3 replicates.

Table 2: Minimum Inhibitory Concentration of various foot extracts of three snail species against bacteria

Species/extract	Diethylether			Acetone			Ethanol		
	E.coli	P. auroginosa	S. aureus	E.coli	P. auroginosa	S. aureus	E.coli	P. auroginosa	S. aureus
	MIC (ug/ml)			MIC(ug/ml)			MIC(ug/ml)		
Physa Acuminata	0.3	0.6	0.3	0.3	1.25	1.25	0.07	0.07	0.03
Physa Fontinalis	0.09	1.25	0.1	0.09	0.6	0.07	0.6	0.03	0.03
Physa Gyrina	2.5	2.5	0.09	2.5	32.5	0.6	0.01	0.01	0.01
C.Petitiana	1.25	2.5	0.1	5	2.5	2.5	1.25	2.5	0.07
C.Acicula	2.5	0.6	1.25	1.25	1.25	0.3	2.5	2.5	0.01
C. Eulima	5	5	2.5	2.5	5	1.25	5	5	0.6

Table 3: Analysis of Variance among snail species, bacterial strains and foot extracts, using Adjusted SS for Test

Source	DF	Seq SS	Adj SS	Adj MS	F-value	P-value
Snail species	5	69.1	69.1	13.8	5.35	0.000
Strains	2	117.4	117.4	58.7	22.74	0.000
Extracts	3	10809.3	10809.3	3603.1	1395.58	0.000

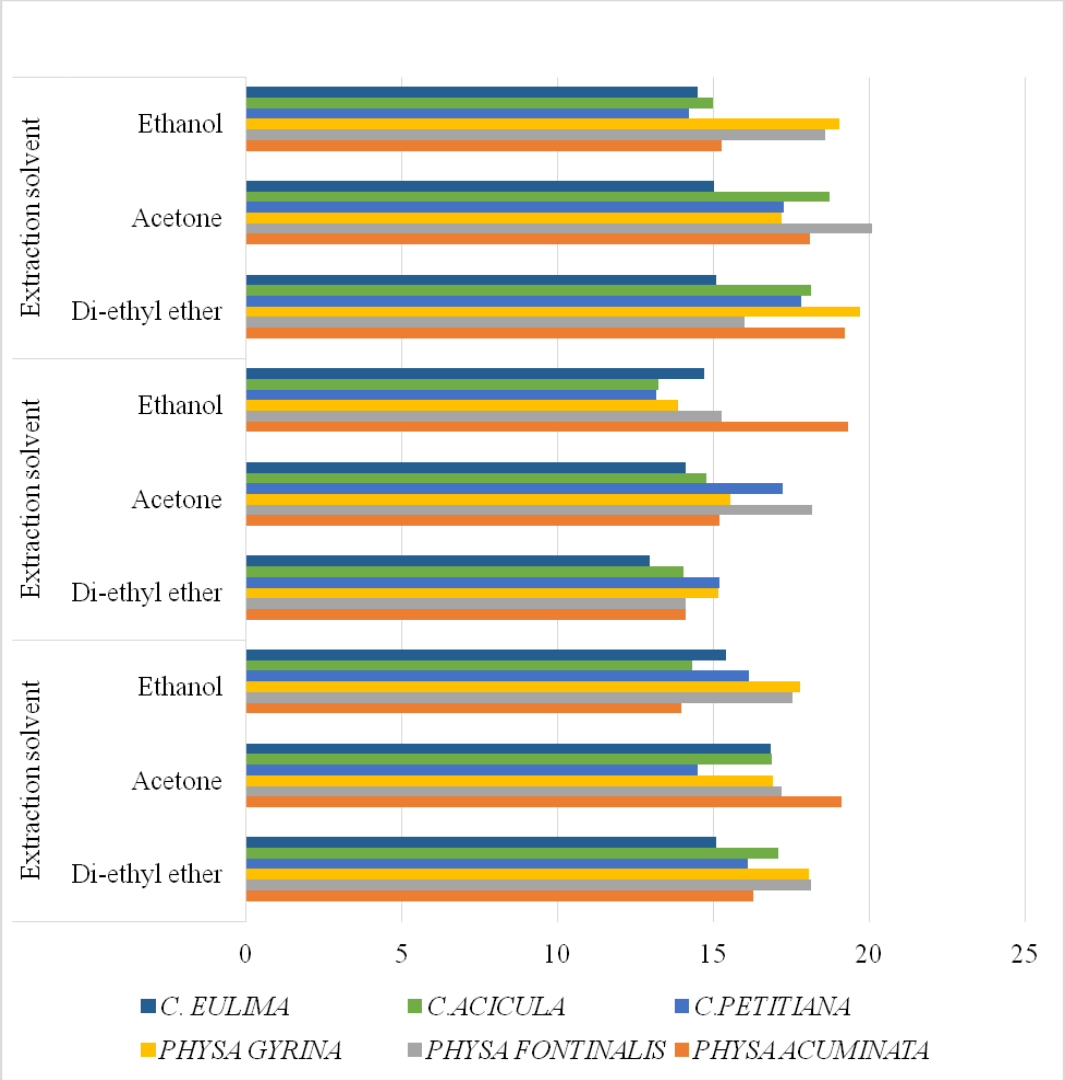

Fig. 1: Antimicrobial activity of various foot extracts of three snail species against bacteria.

Table 4: Multiple Comparisons among means of snail species on the zone of inhibition using Tukey test

(I) Snail Spieces	(J) Snail Spieces	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
PA	PF	-.5074	.33712	.662	-1.4856	.4708
	PG	-.3000	.33712	.948	-1.2782	.6782
	CP	.7630	.33712	.218	-.2152	1.7411
	CA	.9296	.33712	.072	-.0485	1.9078
	CE	1.6370*	.33712	.000	.6589	2.6152
PF	PA	.5074	.33712	.662	-.4708	1.4856
	PG	.2074	.33712	.990	-.7708	1.1856
	CP	1.2704*	.33712	.004	.2922	2.2485
	CA	1.4370*	.33712	.001	.4589	2.4152
	CE	2.1444*	.33712	.000	1.1663	3.1226
PG	PA	.3000	.33712	.948	-.6782	1.2782
	PF	-.2074	.33712	.990	-1.1856	.7708
	CP	1.0630*	.33712	.025	.0848	2.0411
	CA	1.2296*	.33712	.005	.2515	2.2078
	CE	1.9370*	.33712	.000	.9589	2.9152
CP	PA	-.7630	.33712	.218	-1.7411	.2152
	PF	-1.2704*	.33712	.004	-2.2485	-.2922
	PG	-1.0630*	.33712	.025	-2.0411	-.0848
	CA	.1667	.33712	.996	-.8115	1.1448
	CE	.8741	.33712	.108	-.1041	1.8522
CA	PA	-.9296	.33712	.072	-1.9078	.0485
	PF	-1.4370*	.33712	.001	-2.4152	-.4589
	PG	-1.2296*	.33712	.005	-2.2078	-.2515
	CP	-.1667	.33712	.996	-1.1448	.8115
	CE	.7074	.33712	.296	-.2708	1.6856
CE	PA	-1.6370*	.33712	.000	-2.6152	-.6589
	PF	-2.1444*	.33712	.000	-3.1226	-1.1663
	PG	-1.9370*	.33712	.000	-2.9152	-.9589
	CP	-.8741	.33712	.108	-1.8522	.1041
	CA	-.7074	.33712	.296	-1.6856	.2708

Based on observed means.
The error term is Mean Square (Error) = 1.534. *. The mean difference is significant at the 0.05 level.

ANOVA test showed that extracts of snail's foot muscles species antibacterial activity is highly significant against control that showed no activity (table 3).

The posthoc test i.e. tukeys test shows that the foot extracts of the members of the genus Physa showed highly significantly different results from genus Ceciloides. When the means of zone of inhibition were compared for the different solvent used for the extract preparation, it was found that ethanol extracts showed highly significant results when compared to acetone and diethyl ether, while the results were significantly different when the three solvent extracts were compared. Comparing the three strains for the zone of inhibition, it was found that the results were highly significantly different. The results show that the members of the genus Physa are a stronger candidate for having potent bioactive agents in ethanol extracts (table 4; table 5; table 6)

Thin layer chromatography showing refractive index Value

Thin layer chromatography is profiling of extract which

showed best activity. Out of 18 extracts in 3 different solvents 11 extract showed best activity. TLC was done in solvent system of 100% pure n-hexane. The plates developed in solvent system showed purple and yellow spots indicating the presence of proteins when treated with ninhydrin. Rf value for *P. fontinalis* showed highest activity against *S. aureus* in acetone extract 0.75 cm indicating protiens. As followed Rf value for *P. gyrina* 0.76 cm, for *P. acuta* 0.71cm.Similarly Rf value for *P. gyrina* in ethanol extract is 0.56 cm, for *P. acuta* 0.52 cm, for *P. fontinalis* 1 cm. In diethylether extract Rf value for *P. gyrina* is 0.75 cm, for *P. fontinalis* it is 0.73cm, for *P. acuta* it is 0.74 cm.

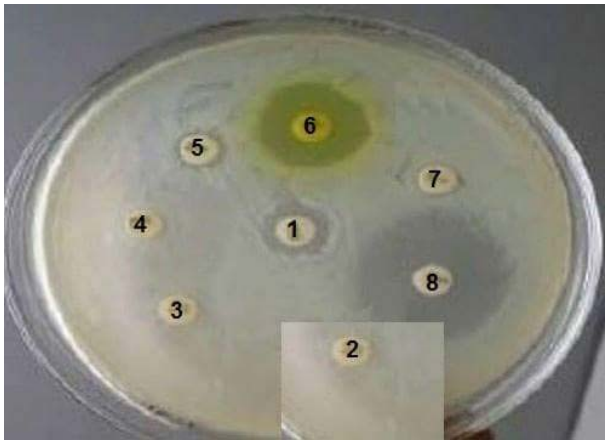

Fig. 2: Zone of inhibitions of *P.fontinalis* against *S.aureus* in acetone extract

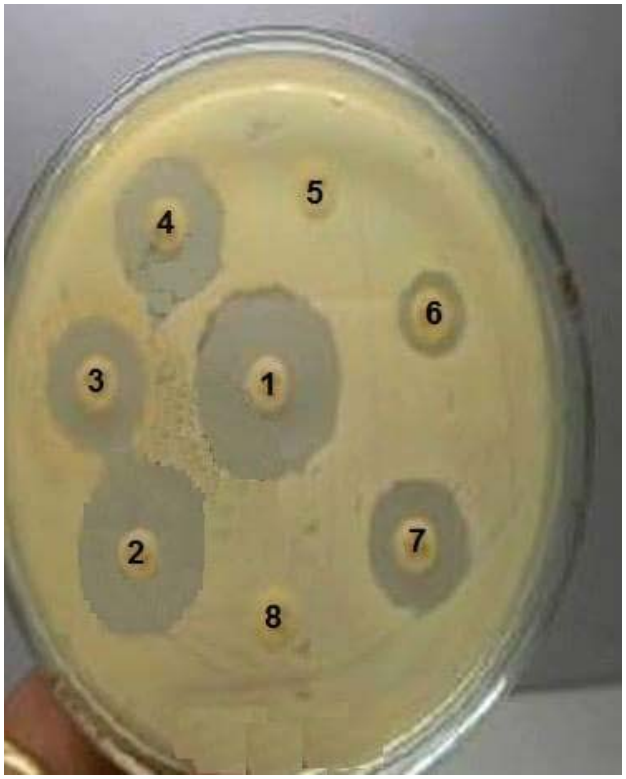

Fig. 3: Zone of inhibitions *P. gyrina* against in *E. coli* in ethanol extract

Three extract of cecilioides also showed good antibacterial activity. Rf value for Cecilioides species is 0.50cm for *C. petitiana* and 0.22 cm C. *acicula* in ethanol extract. As followed in ethanol extract *C. acicula* Rf value is 0.42cm (table 7; fig. 5)

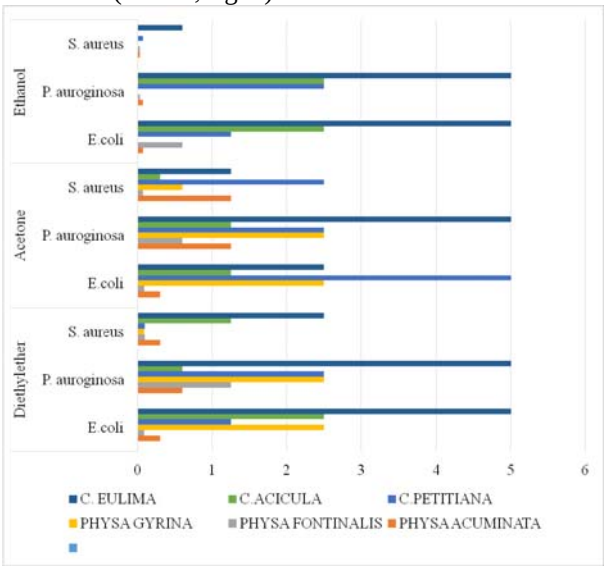

Fig. 4: Minimum Inhibitory concentration of various foot extracts of three snail species against bacteria.

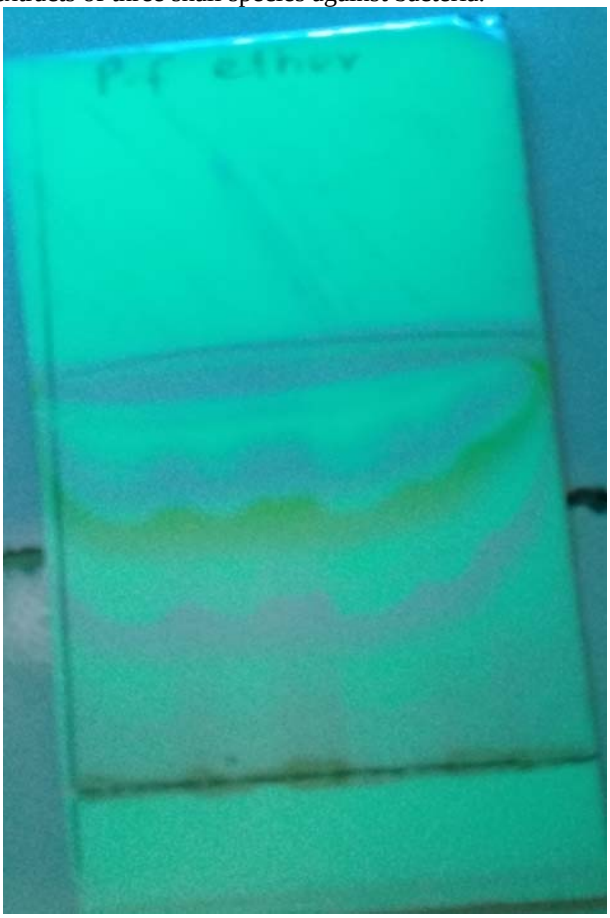

Fig. 5: Thin Layer Chromatography of *Physa fontinalis* foot extract in Ether

Table 5: Comparisons among means of zone of inhibition with various extraction solvent using Tukey test

(I) Extract	(J) Extract				95% Confidence Interval	
		Mean Difference (I-J)	Std. Error	Sig.	Lower Bound	Upper Bound
Diethylether +DMSO	Aceone +DMSO	-.5852*	.23838	.041	-1.1517	-.0187
	Ethanol +DMSO	.7241*	.23838	.008	.1576	1.2906
Acetone +DMSO	Diethylether +DMSO	.5852*	.23838	.041	.0187	1.1517
	Ethanol +DMSO	1.3093*	.23838	.000	.7428	1.8758
Ethanol +DMSO	Diethylether +DMSO	-.7241*	.23838	.008	-1.2906	-.1576
	Acetone +DMSO	-1.3093*	.23838	.000	-1.8758	-.7428

Table 6: Comparisons among means of the zone of inhibition among various strains using Tukey test

(I) Strain	(J) Strain				95% Confidence Interval	
		Mean Difference (I-J)	Std. Error	Sig.	Lower Bound	Upper Bound
E. coli	*P. auroginosa*	1.5093*	.23838	.000	.9428	2.0758
	S. aureus	-.8704*	.23838	.001	-1.4369	-.3039
P. auroginosa	*E- coli*	-1.5093*	.23838	.000	-2.0758	-.9428
	S. aureus	-2.3796*	.23838	.000	-2.9461	-1.8131
S. aureus	*E. coli*	.8704*	.23838	.001	.3039	1.4369
	P. auroginosa	2.3796*	.23838	.000	1.8131	2.9461

Table 7: Refractive Index values in 100% n-hexane and 100% Ethyl acetate for acetone, di-ethyl ether and ethanol foot extract of species

No of species	Species name	Rf value of acetone extract in Ethyl acetate	Rf value of di ethyle ether extract in n-hexane	Rf value of ethanol extract in n-hexane
1	*P. fontinalis*	0.75	0.73	0.56
2	*P. gyrina*	0.76	0.76	0.52
3	*P.acuta*	0.71	0.74	0.1
4	*C.petitiana*	_	_	0.50
5	*C.acicula*	_	0.42	0.22

DISCUSSION

Highest antibacterial activity was recorded in *P. fontinalis* in acetone extract against *S. aureus* by using disc diffusion assay. Maximum zone of inhibition was recorded as 20.01 mm at 10mg/ml. These results are very close to the findings of Bensig *et al*. (2014) who revealed that the methanolic extracts of body of *Helico styladaphnis* across all ranging from 0.25mg/L to 1mg/L were able to show an inhibitory action ranging from 5-25mm zone of inhibition against the bacterial activity, however diluting extract generally weakens the antimicrobial activity of the extract (Jensen *et al*., 1996). Similary the tissue sample extracts of the gastropod species *H. dividis* showed a range of zone of inhibition from 2mm to 10mm against various human and fish pathogens with a concentration of 30µL with methanolic extract being the most effective. The antimicrobial activity of diethyl ether extract of *Pomacea insularium* has been found extremely effective against *Streptococcus* species (37.16 mm) (Lekshmi *et al*. 2015). The bioactivity against various bacteria and viruses have been reported in heamolymph of various molluscan species including mussels, oysters, sea slugs, sea hares (Nakamura *et al*., 1988; Maktoob and Ronald, 1997; Mitta *et al*., 1999; Olicard *et al*., 2005; Gueguen *et al*., 2006; Roch *et al*., 2008). The disc diffusion assay revealed more activity against *Pseudomonas* sp. than against *Streptococcus* sp. and *Staphylococcus* sp. (Etim *et al*., 2016). Strong antibacterial activity of the ethanolic body extracts of *Babylonia spirata* have been observed against *S. typhi, E. coli, P. vulgaris,* and *K. pneumoniae* (Anand *et al*., 1997) and *P. aeruginosa* (Periyasamy *et al*., 2012) due to the presence of proteins that are shighly potent antibiotics (Kuppusamy *et al*., 2016).

The foot extracts of *P. acuminata* and *P. fontinalis* in all the three solvents are found quite effective against all the three bacterial strains. Among all the extracts examined in this study, di ethyl ether and acetone extract showed the least activity; it inhibited the organisms in high concentration compared to other extracts. MIC 0.09 µl

was recorded in *P. fontinalis* against *E.coli* followed by *P. gyrina* 0.09 µl against *S .aureus* (table 2; fig. 2).The results show that the said species and various others snail species are strong candidates for the exploration of bioactive compounds having antibacterial activity which are in conformation with previous results stating that the mucous of *A. marginata* (Etim *et al*., 2016) *T. tentorium* (Anbuselvi *et al*., 2009) *T. radiatus* (Gananambal *et al*., 2005) has strong inhibitory action against various species of *Pseudomonas*, *Streptococcus*, *Staphylococcus*. Similar results have been reported from other marine molluscan body extracts (Anand and Edward, 2002) including *Sepia sp.* and *Loligo sp* that inhibit the growth of *P. aeruginosa* and *E. coli* (Degiam and Abas, 2010). Approximately more than 1,100 antibiotic substances have been isolated from invertebrates. Among these, 50 have found widespread use in the prevention and treatment of bacterial diseases in animal and man (Gale and Kiser (1967).

Lekshmi *et al*, (2015) develop TLC plates for hemolymph of snail to check the presence of compound. These TLC plates developed in the solvent system and they showed the spots of yellow colour which indicate the presence of proteins. These results concide with the results of present study in which all three species of Genus Physa gives purple spot on TLC plate when subjected in the ethyl acetate solvent.

The molecular weight of crude protein from *Babylonia spirata* was ranged from 2-110kDa on SDS PAGE (Periyasamy *et al*., 2012). In result of the present study clearly showed that, 2.6mg/ml protein concentrations were obtained in digested protein hydrolysate. Molecular weight ranging from 40 to 200kDa was found in the protein hydrolysate of *B. spirata* which act as bioactive compounds for various biological activities. Marine molluscs have been found to produce a great diversity of novel bioactive compounds and to be a potential source for new drug discovery. There has been a remarkable progress in the prevention; control and even eradication of infectious diseases with improved hygiene and development of antimicrobial compounds (Kuppusamy, 2016). Various bacterial species have been implicated in wound infections, some of which have been identified as *Staphylococcus aureus*, Coagulase-negative *Staphylococci*, *E. coli*, *P. aeruginosa* (Etim *et al*. 2016).

CONCLUSION

We found that the members of the genus Physa are strong candidates with potent bioactive proteins and must be tested further for the natural drug discovery.

REFERENCES

Abiona D, Adedapo Z and Suleiman M (2015). Proximate Analysis, Phytochemical Screening and Antimicrobial Activity of Baobab (Adansoniadigitata) leaves. *IOSR JAC*, **8**(5): 60-65.

Anand TP and Edward JK (2002). Antimicrobial activity in the tissue extracts of five species of cowries Cypraea spp. (Mollusca: Gastropoda) and an ascidian *Didemnum psammathodes* (Tunicata: Didemnidae). Anand TP, Rajaganapathi J and Edward JK (1997). Antibacterial activity of marine molluscs from Portonovo region. *Indian j. mar. sci.,* **26**: 206-208.

Andrews JM (2001). Determination of minimum inhibitory concentrations. *J. Antimicrob. Chemother.*, **48**(suppl 1): 5-16.

Anbuselvi SC, Chellaram S, Jonesh L Jayanthi and JKP Edward (2009). Bioactive potential of coral associated gastropod, Trochus tentorium of Gulf of Mannar, Southeastern India. *J. Med. Sci.*, **9**(5): 240-244.

Ali RA (2005). *A Study on the Occurrence of Some Mollusca Species in Sugarcane Fields.* MPhil Thesis, Department of Zoology and Fisheries, University of Agriculture, Faisalabad.

Altaf J, Qureshi NA and Siddiqui MJI (2017). Terrestrial snails as bioindicators of environmental degradation. *J. Bio. Env. Sci.*, **10**(1): 253-264.

Chellaram CMEG, Gnanambal K and Edward JK (2004). Antibacterial activity of the winged oyster *Pteria chinensis* (Pterioida: Pteridae). *Indian j. mar. sci.*, **33**: 369-372.

Degiam ZD and AT Abas (2010). Antimicrobial activity of some crude marine Mollusca extracts against some human pathogenic bacteria. *TQMJ.*, **4**(3): 142-147.

De Smet L, Dimitrov I, Debyser G, Dolashka-Angelova, P, Dolashki A, Van Beeumen J and Devreese B (2011). The cDNA sequence of three hemocyanin subunits from the garden snail Helix lucorum. *Gene*, **487**(2): 118-128.

Dolashka-Angelova P, Stefanova T, Livaniou E, Velkova L, Klimentzou P, Stevanovic S, Salvato B, Neychev H and Voelter W (2008). Immunological potential of *Helix vulgaris* and *Rapana venosa* hemocyanins. *Immunol. Investig.,* **37**(8): 822-840.

Dolashka P, Velkova L, Iliev I, Beck A, Dolashki A and Yossifova L *et al* (2011). Antitumor Activity of Glycosylated MolluscanHemocyanins via Guerin ascites tumor. *Immunol. Investig.*, **40**(2): 130-149.

Etim LB, Aleruchi C and Obande GA (2016). Antibacterial properties of snail mucus on bacteria isolated from patients with wound infection. *British Microbiol. Res. J.*, **11**: 1-9.

Gale GO and Kiser JS (1967). Division of microbiology: antibiotic resistance theory and practice. *Trans.* N. Y. *Acad. Sci.*, **29**(7): 960-968.

Gnanambal KME, Chellaram C and Patterson JK (2005). September. Antibacterial activity of whole body extracts of *Trochus radiatus* (Mollusca: Gastropoda). *In*: Proceedings of the National Seminar on Reef Ecosystem Remediation, SDMRI Research Publication, **9**: 182-186.

Gerdol M, De Moro G, Manfrin C, Venier P and Pallavicini A (2012). Big defensins and mytimacins, new AMP families of the Mediterranean mussel *Mytilus galloprovincialis. Dev Comp Immunol*, **36**(2): 390-399.

Gueguen Y, Bernard R, Julie F, Paulina S, Delphine DG, Franck V, Philippe B and Evelyne B (2009). Oyster hemocytes express a proline-rich peptide displaying synergistic antimicrobial activity with a defensin. *MOL IMMUNOL*, **46**(4): 516-522.

Jensen PR, Harvell CD, Wirtz K and Fenical W (1996). Antimicrobial activity of extracts of Caribbean gorgonian corals. *MAR BIOL*, **125**(2): 411-419.

Kahlown MA, M Ashraf, M Hussain, A Salam and AZ Bhatti (2006) Impact Assessment of Sewerage and Industrial Effluents on Water Resources, Soil Crops and Human Health in Faisalabad, Research Report-6. PCRWR.

Kuppusamy A and Ulagesan S (2016). Antimicrobial activity of protein hydrolysate from marine molluscs *Babylonia spirata* (Linnaeus, 1758). *J Appl Pharm Sci*, **6**(7): 073-077.

Laxminarayan R, Duse A, Wattal C, Zaidi AK, Wertheim, HF, Sumpradit N, Vlieghe E, Hara GL, Gould IM Goossens H and Greko C (2013). Antibiotic resistance the need for global solutions. *Lancet Infect Dis*, **13**(12): 1057-1098.

Lekshmi NCJP, Viveka S, Anusha S, Jeeva S, Brindha R and Selva Bharath M (2015). Antibacterial activity of fresh water crab and snail and isolation of antibacterial peptides from haemolymph by SDS PAGE. *Int. J. Pharm. Pharm. Sci.*, **7**(1): 109-114.

Li H, Parisi, MG, Parrinello N *et al* (2011). Molluscan antimicrobial peptides, a review from activitybased evidences to computer-assisted sequences. *Invertebrate Surviv. J.*, **8**: 85-97.

Maktoob A and Ronald HT (1997). Handbook of natural products from marine invertebrates. Phyllummollusca Part. 1, Harwood Academic Publishers, Newark, USA, pp.1-288.

Mitta G, Hubert F, Noel T and Roch P (1999a). Myticin a novel cysteinerich antimicrobial peptide isolated from haemocytes and plasma of the mussel *Mytilus galloprovincialis. Eur. J. Biochem.*, **265**: 71-78.

Nakamura T, Furunaka HTTM, Miyata T, Tokunaga F, Muta T, Iwanaga S, Niwa M, Takao T and Shimonishi Y (1988). Tachyplesin, a class of antimicrobial peptide from the hemocytes of the horseshoe crab (*Tachypleus tridentatus*). Isolation and chemical structure. *J Biol Chem*, **263**(32): 16709-16713.

Olicard C, Renault T, Torhy C, Benmansour A and Bourgougnon N (2005). Putative antiviral activity in hemolymph from adult Pacific oysters, *Crassostrea gigas*. *Antiviral* Res , **66**(2-3): 147-152.

Pelczar MJ, Chan ECS and Krieg NR (1988). Control of microorganisms, the control of microorganisms by physical agents *Microbiology*, **469**: 509.

Periyasamy N, Srinivasan M and 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.

Proksch P, Edrada R and Ebel R (2002). Drugs from the seas–current status and microbiological implications. *Appl. Microbiol. Biotechnol*, **59**(2-3): 125-134.

Subba Rao NV (2003). Indian sea shells (part 1) Polyplacophora and gastropoda. *Rec. Zool. Sur. India*, **416**: 96.

Rahman MSU (2010). Biodiversity of the Snails from the Agro-Ecosystem of Tehsil Faisalabad City. B. S Thesis, Government College University, Faisalabad.

Roch P, Yang Y, Toubiana M and Aumelas A (2008). NMR structure of mussel mytilin and antiviral antibacterial activities of derived synthetic peptides. *Dev. Comp. Immunol.*, **32**: 227-238.

Schwalbe R, Steele-Moore L and Goodwin AC eds., (2007). Antimicrobial susceptibility testing protocols. CRC Press.

Soderhall K (2011). Invertebrate Immunity. Springer Science & Business Media, New York, USA, 2011.

Suarez-Jimenez GM, Burgos-Hernandez A and Ezquerra-Brauer JM (2012). Bioactive peptides and depsipeptides with anticancer potential: Sources from marine animals. *MAR DRUGS* **10**(5): 963-986.