International Journal of Life Sciences
International Peer Reviewed Open Access Journal

Special Issue A 13, December 2019
ISSN:2320-7817(p) 2320-964X(0)

Original Article Open Access

Biochemical efficacy of *Ircinia fusca* marine sponge of Ratnagiri Coast (MS) India

Lakwal VR*[1], Shaikh SA[2], Kharate DS[3], Kalse AT[4] and Mokashe SS[5]

*[1,4] P.G.Department of Zoology, Nanasaheb Yashwantrao Narayanrao Chavan Arts, Science and Commerce College, Chalisgaon, Jalgaon (MS) - 424101
[2]Department of Zoology, Sundarrao Solanke Mahavidhyalay Majalgaon, Beed – 431131
[3]Department of Zoology, Vinayakrao Patil College, Vaijapur, Auranagabd – 423701
[5]Department of Zoology, Dr. Babasaheb Ambedkar Marathwada University, Aurangabad - 431001
*Corresponding Author: vijaylakwal02@gmail.com

Manuscript details:

Available online on
http://www.ijlsci.in

ISSN: 2320-964X (Online)
ISSN: 2320-7817 (Print)

Editor: Dr. Arvind Chavhan

Cite this article as:
Lakwal VR, Shaikh SA, Kharate DS, Kalse AT and Mokashe SS (2019) Biochemical efficacy of *Ircinia fusca* marine sponge of Ratnagiri Coast (MS) India, *Int. J. of. Life Sciences*, Special Issue, A13: 1-8.

ABSTRACT

The present investigation was made to understand biochemical efficacy (antimicrobial properties and biochemical profile) of *Ircinia fusca* marine sponge collected from Ratnagiri coast. Extraction was done using Hexane, Chloroform, Acetone and Methanol. The crude extracts were tested against known microbial strain by using agar well diffusion method. Acetone showed strong positive antibacterial activity against *Escherichia coli, Staphylococcus aureus, Salmonella typhi, Bacillus subtilis*, whereas, positive against methanol, chloroform and hexane. The methanol and acetone extracts showing strong positive antifungal activity against *Penicillium sp., and Fusarium sp.,* and weak positive activity against *Aspergillus sp., and Alternaria sp,.* Biochemical screening of hexane and chloroform extract revealed the presence of protein and amino acids, steroids, carbohydrates, fats and fixed oil and absence of alkaloids, glycosides, tannins and flavonoids. The acetone and methanol extract contain alkaloids, glycosides, tannins, flavonoids, proteins and amino acids, steroids, carbohydrates, fats and fixed oils.

Key words: *Ircinia fusca,* biochemical screening, antimicrobial activity, Hexane, Chloroform, Acetone, Methanol.

INTRODUCTION

Sponges are simple, multicellular, sessile animals with no true tissue layers or organs (Bergquist 1938). This rocky shore area is directly exposed to sea and inhabited by diverse flora and fauna. Sponges are the most primitive multicellular animals that have existed for more than 800 million years. The sponges (Porifera), being evolutionarily ancient inhabit every type of marine benthic environment (Radjasa, *et al.,* 2007). They inhabit from polar sea (Dayton *et al*., 1974) to temperate and tropical waters (Reiswig, 1973; Wenner *et al.,* 1983) and are often more abundant and diverse in the tropics than stony and soft corals (Targett and Schmahl, 1984).

Ircinia fusca (Carter 1880) is a jet black, thick encrusting to massive, sometimes irregular sponge found commonly on the intertidal rock pools of Ratnagiri, west coast of India. They have presence novel bioactive compounds with more than 200 new metabolites reported on each year. The compounds derived from has a wide range of chemical classes (alkaloids, peptides, terpenoids, and polyketides) with an equally wide ranges of biotechnologically relevant properties e.g., anticancer, antibacterial, antifungal, antiviral, anti-inflammatory and antifoulings (Blunt, *et al.,* 2005, Blunt, *et al.,* 2006). Sponges have been considered as a gold mine for the chemists. Bioactive compounds have been isolated from some sponge and a first compounds was made available on market in 2004, the secondary metabolites of marine sponges are rich source of pharmacologically active compounds that can potentially be used as medicines to cure human diseases (Azevedo, *et al*., 2008).

The sponges known to produce the largest number and diversity of secondary metabolites as compare to other marine invertebrates. Although the functions of these secondary metabolites are largely unknown, there is some evidence that they provide chemical defence against predators (Chanas *et al.*, 1997). The first report of antimicrobial activity of sponge extracts was by Nigrelli (Nigrelli, 1959). The antimicrobial activity of two marine sponge species such as *Psammaplysilla purpurea* and *Ircinia ramose,* which were collected from the Gulf of Mannar, India was analysed their antibacterial, antifouling activities against various pathogenic bacteria (Kanagasabhapathy *et al.,* 2004). The sponge *Ircinia ramosa* has also been shown to possess antiviral, CNS stimulatory (Parulekar and Shirvoikar, 1991), and antialgal properties (Mokashe *et al.,* 1994).The present study describes the biochemical efficacy (antimicrobial properties and biochemical profile) of the crude extract of *Ircinia fusca* against the pathogenic microbes.

MATERIAL METHODS

Collection of Sponges, Sample Preparation and Extraction

The sponge *Ircinia fusca* were collected from intertidal pools of Ratnagiri Coast (16º59'N 73º16'E), (MS) India. The sponge collection was not harmful to an ecosystem. Identified sponge tissues samples were incised out and washed with sea water, air dried and chopped into small size and extracted with 200 ml hexane, chloroform, acetone and methanol for about 15 days. After 15 days the extract was filtered through Whatmann filter paper (No: 2) and Solvents were removed by rotary vacuum evaporator (Buchi type Superfit, Bangalore)) under reduced pressure so as to get the crude sponge extract. Then desalting process and make it pure extract. The concentrated crude extract was used for antimicrobial study.

Antimicrobial activity

For the antimicrobial activity 4 species of bacterial and 4 species of fungi were selected. The bacterial and fungal strains were obtained from Government Institute of Science, Aurangabad, (MS) India. *Escherichia coli, Salmonella typhi,* (Gram negative bacteria) *Bacillus subtilis, Staphylococcus aureus,* (Gram positive bacteria) strains were used. *Aspergillus sp., Penicillium sp., Alternaria sp. and Fusarium sp.,* were used as fungal test microorganisms.

Antibacterial activity by well assay method

Assays were performed according to the standard guidelines of the National Committee for Clinical Laboratory Standards (NCCL, 1990) using a modified Kirby–Bauer well assay method. All bacteria were stored at −20°Cuntil use. Cells were grown in Muller Hinton broth and were transfer to Muller Hinton agar. Broth cultures were swabbed onto agar medium to achieve a lawn of confluent bacterial growth separately for each strain. Stainless steel borer use to make well. Five wells were bored in each plate. 100 µg/ml extracts loaded on the well and find out inhibitory effect. Discs of Streptomycin (25µg/ml) were used as positive control. The plates were incubated at 37°Cfor 24 hrs. The growth of bacterias around each well was observed carefully and the zone of inhibition around each well was measured using a Hi-media zone reader triplicate plates were maintained for each test.

Antifungal activity by well assay method

Assays were performed according to the standard guidelines of the National Committee for Clinical Laboratory Standards (NCCL, 1990) using a modified Kirby–Bauer well assay method. All fungi were stored at −20°C until use. Cells were transfer to Sabouraud dextrose. The fungal cultures were maintained in 0.2% Sabouraud dextrose medium. Each fungal inoculum was applied on plate and evenly spread on Sabouraud dextrose agar using a sterile cotton swab. A sterile stainless-steel borer (6 mm) used to make well in the medium. Five wells were bored in each plate. The

sponge extract 100 µg/ml was loaded into the well and check the inhibitory potential. Discs of the Fluconazole were used as the positive control. The plates were incubated at 28°Cfor 48 hrs. The growth of fungi around each well was observed carefully and the diameter of the zone of inhibition around each well was measured using a Hi-media zone reader Triplicate plates were maintained for each test.

Preliminary screening of sponges for chemical constituents

It involves testing of different extracts of *Ircinia fusca* for their contents of different classes of compounds. The qualitative chemical tests for various bioconstituents were carried out for all the extracts of *Ircinia fusca* described by (Harborne, 1998).The sponge extracts were analysed for the presence of various compounds as described by Okawori *et al.,* 2008.

1. Detection of alkaloids

Mayer's Test: Extracts were treated with Mayer's reagent (Potassium Mercuric Iodide). Yellow coloured precipitation indicates the presence of alkaloids.

Wagner's Test: Extracts were treated with Wagner's reagent (Iodine in Potassium Iodide). Brown/reddish precipitation indicates the presence of alkaloid.

Dragendroff's Test: Extracts were treated with Dragendroff's reagent (solution of Potassium Bismuth Iodide). Red precipitate indicates the presence of alkaloid.

Hager's Test: Extracts were treated with Hager's reagent (saturated picric acid solution). Yellow coloured precipitate showed presence of alkaloids.

2. Detection of glycosides

Legal's Test: Extracts treated with sodium nitroprusside and sodium hydroxide. Pink to blood red colours indicate the presence of cardiac glycosides.

3. Detection of tannins

Gelatin Test: Extract with 1% gelatin solution containing sodium chloride was added. White precipitate indicate the presence of tannins.

Ferric Chloride Test*:* With 1% ferric chloride solution the extract gives blue, green, or brownish green colour indicating the presence of tannins.

4. Detection of flavonoids

Alkaline Reagent Test: Extracts were treated with few drops of sodium hydroxide solution. Intense yellow colour, which becomes colourless on addition of dilute acids, indicates the presence of flavonoid.

Lead acetate Test: Extracts were treated with few drops of lead acetate solution. Yellow colour precipitations indicate the presence of flavonoid.

Shinoda Test: 2-3 ml of extract, a piece of magnesium ribbon and 1 ml of conc. hydrochloric acid was added. Pink or red coloration of the solution indicates the presence of flavonoids.

Zinc Hydrochloride Test: To the test solution, add a mixture of zinc dust and conc. Hydrochloric acid. It gives red colour after few minutes.

5. Detection of proteins and amino acids

Xanthoproteic Test: The extracts were treated with few drops of conc. Nitric acid. Yellow colour indicate the presence of protein.

Ninhydrin Test: In the extract 0.25% w/v ninhydrin reagent was added and boiled for few minutes. Blue colours indicate the presence of amino acids.

Detection of saponins

Foam Test: Small amount of extract was shaking with 2 ml of water. If foam formation persists for ten minutes it indicates the presence of saponin.

Sterols and Terpenoids

Salkowski's Test: Extracts were treated with few drops of Conc. Sulphuric acid, Red colour at the lower layer indicate presence of steroids and formation of yellow colour at the lower layer indicates the presence of terpenoid.

6. Detection of carbohydrates

Molisch's Test*:* Extracts were treated with 2 drop of alcoholic α-naphthol solution in a test tube. Violet ring at the junction indications the presence of Carbohydrate.

Benedict's Test: Filtrates were treated with Benedict's reagent and heated gently. The formation of orange red precipitation indicate the presence of reducing sugar.

Fehling's Test: Filtrates were hydrolysed with dil. HCl, neutralize alkali and heated with Fehling's A and B

solution. Red precipitations indicate the presence of reducing sugar.

Selwanoffs Test: One half ml of a sample solution is placed in a test tube. 2 ml of selwinoffs reagent (a solution of resorcinol and HCL) is added. The solution is heated with boiling water bath for two minutes. A positive test is indications by the formation of a red product.

Camnelisation Test: 1 ml extract were treated with strong sulphuric acid gives a burning sugar smell. This indicates the presence of carbohydrates.

7. Fats and Fixed Oils

Stain Test: Small amount extract was pressed between two filter papers. Oily stain appears on filter paper indicates the presence of fixed oil.

Saponification test:- Add a few drops of 0.5N of alcoholic potassium hydroxide to small quantities of extracts along with a drop of Phenolphthalein separately and heat on a water bath for 1-2 hrs. Soap indications the presence of fixed oils and Fat.

RESULTS & DISCUSSION

The antibacterial activity of *Ircinia fusca* in the agar well diffusion method is given in Fig. 1. The acetone extracts of *Ircinia fusca* exhibited strong positive inhibitory activity towards human pathogenic bacteria such as *E. coli, Salmonella typhi,* (Gram negative bacteria) *Bacillus subtilis, Staphylococcus aureus,* (Gram positive bacteria), Whereas in methanol, chloroform and hexane shows the positive inhibitory activity against all pathogens depicted in table 1., Fig. 2 showing the antifungal activity of *Ircinia fusca* by agar well diffusion method. The hexane and chloroform depicted weak activity against all pathogenic fungus. The methanol and acetone extracts of *Ircinia fusca* showing strong inhibitory effects against pathogenic fungus such as *Penicillium sp., and Fusarium sp.,* and weak activity was seen in *Aspergillus sp., and Alternaria sp.* Showed in table 2.

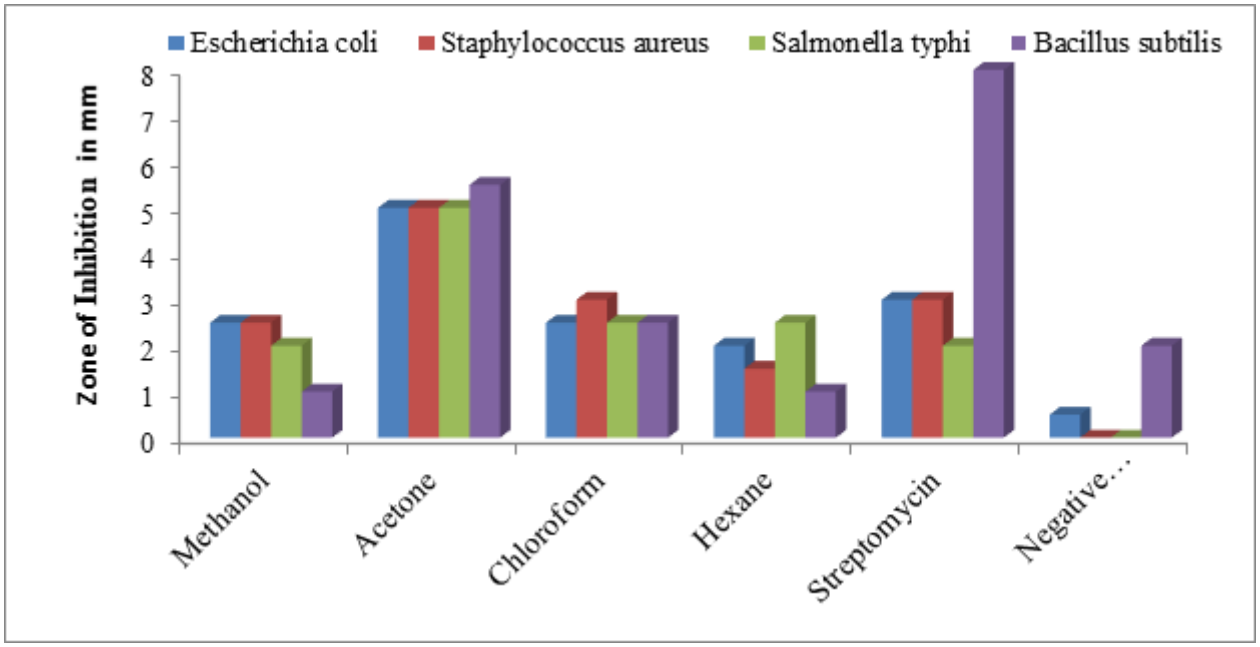

Table 1: **Antibacterial activity of marine sponge *Ircinia fusca* extract against bacteria**

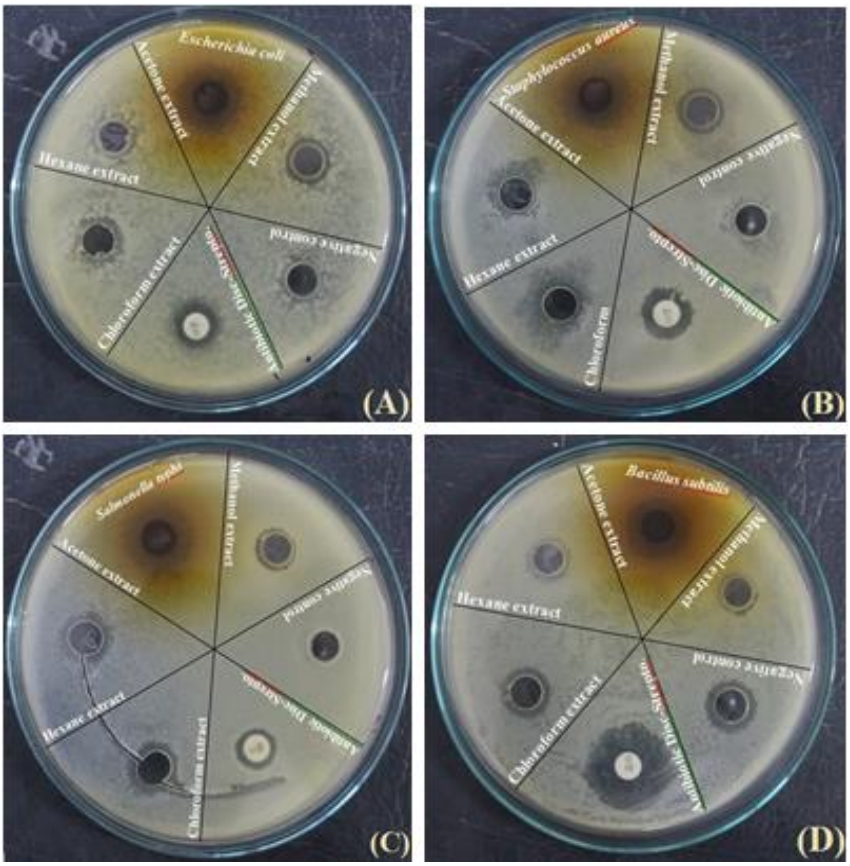

Fig.1. Antibacterial activity *A) Escherichia coli, B) Staphylococcus aureus,C) Salmonella typhi, and D) Bacillus subtilis*

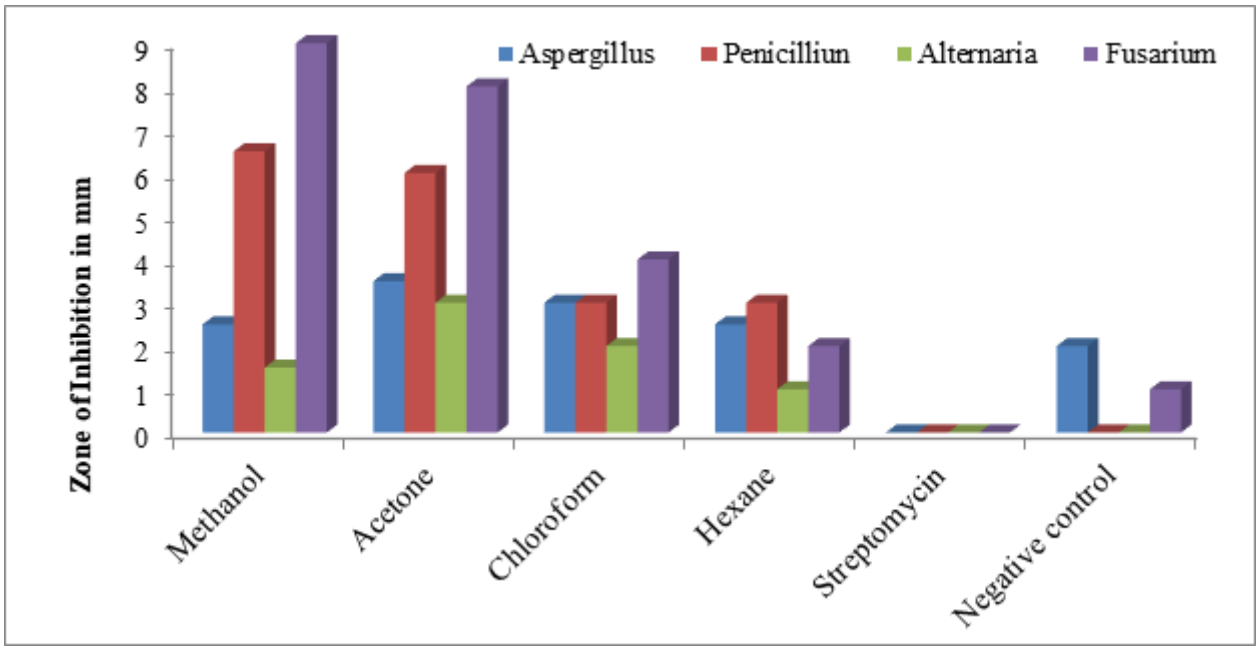

Table 2: **Antifungal activity of marine sponge *Ircinia fusca* extract against fungus**

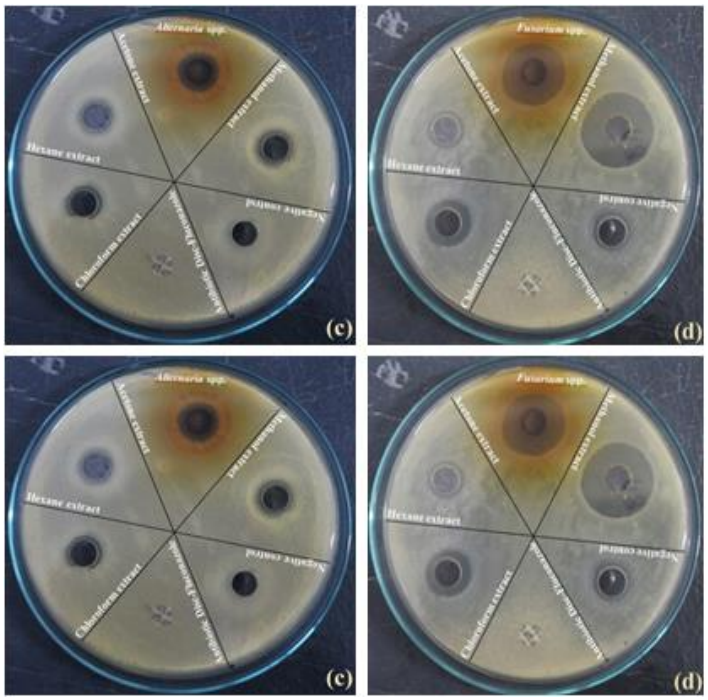

Fig.2. Antifungal activity: *a) Aspergillus sp., b) Penicillium sp., c) Alternaria sp., and d) Fusarium sp.*

Table 3: Showing biochemical tests for various extracts

Biochemicals		Hexane	Chloroform	Acetone	Methanol
Alkaloids	Mayer's Test	-	-	+	-
	Dragendorff's Test	-	-	++	+
	Wagner's Test	+	+	+	+
	Hager's Test	-	-	+	++
Glycosides	Kedde Test	-	-	++	-
	Legal's Test	-	-	-	-
Tannins	Gelatin Test	-	-	-	+
	Ferric Chloride Test	-	-	+	+
Flavonoids	Shinoda Test	-	-	-	-
	Zinc Hydrochloride Reduction Test	+	-	-	-
	Lead Acetate Test	-	+	++	+
	Alkaline Reagent Test	+	-	+	+
Proteins and Amino Acids	Xanthoproteic Test	+	+	+	+
	Millon's Test	-	-	-	-
	Ninhydrin Test	-	+	-	++
Sterol and Terpenoids	Salkowski test	+	+	+	++
Carbohydrates	Molisch's test	++	++	++	++
	Benedict's test	-	-	-	-
	Camnelisation	+	+	+	+
	Selwinoff's test	-	-	+	-
	Fehling's test	-	++	++	-
Fats & Fixed Oils	Stain test	+	+	+	+
	Saponification test	+	+	++	+

(-) No activity, (+) Positive, (++) Strongly Positive

Table 3 depicted the various biochemical present in different extracts. The hexane and chloroform extract contain alkaloids, flavonoids, protein and amino acids, steroids, carbohydrates, fats and fixed oil. The acetone and methanol extract contain alkaloids, glycosides, tannins, flavonoids, proteins and amino acids, steroids, carbohydrates, fats and fixed oils.

In the present study the extracts of the sponge *Ircinia fusca* showed antimicrobial action against the bacteria and fungi. Crude extracts of the *Ircinia fusca* demonstrated good antimicrobial activity against eight microbes (Sharad, *et al.,* 2015). *Ircinia fusca* also possessed an important antimicrobial compound such as 2-Methoxy-1, 4-Benzenediol identified through a GC-MS analysis (Sujatha, *et al.,* 2014). *Ircinia fusca* report one new pyrrole derivative and also exhibits anti-mycobacterial activity (Srinu, *et al.,* 2017). The sponges shows wide spectrum of antibacterial efficacy and exhibited the growth of all the test bacteria. The reports on antibacterial activity of sponges revealed their activity on gram positive bacteria. Various studies have confirmed the predominance of gram negative producers in the marine environment (Sakemi, *et al.,* 1988).

Marine sponge *Aplysina cavernicola* produces the aeroplysinin, aerthionin derivatives, with some antibiotic activity against *Bacillus subtilis* and *Proteus vulgaris* (Thakur and Anil, 2000). They have also confirmed that the sponge species of the southern Eastern Peninsular Indian Coast are the ideal candidates for the production of various antimicrobial (bacterial and fungal) and antifouling drugs (Selvin and Lipton, 2004, Kanagasabhapathy *et al.,* 2004). Hence, the present results profounded the promising antimicrobial activity of *Ircinia fusca* against eight active pathogenic strains. The study shows that *Ircinia fusca* possessed excellent source of antimicrobial properties and secondary metabolites.

CONCLUSION

The present investigation reveals that the marine sponges *Ircinia fusca* shows the potential source for the antimicrobial and biochemical properties. The methanol and acetone depicted strong positive antimicrobial activity. It may be due to the presence of alkaloids, glycosides, tannins, flavonoids, proteins and amino acids, steroids, carbohydrate, fats and fixed oil. The hexane and chloroform showed weak positive antimicrobial activity because absence of alkaloids, glycosides, tannins and flavonoids. The investigation indicated that *Ircinia fusca* remain an interesting source for antimicrobial activity and also suggest that could be a good source of the secondary metabolite. However it required further investigation for isolation of pure compound.

Acknowledgement:
The authors are grateful to the DST/SERB project for financial assessment and the Head, Department of Zoology, Dr.Babasaheb Ambedkar Marathwada University, Aurangabad for providing necessary laboratory facility.

Conflicts of interest: The authors stated that no conflicts of interest.

REFERENCES

Azevedo LG, Peraza GG, Lerner C, Soares A, Murcia N and Muccillo BA (2008) Investigation of the anti-inflammatory and analgesic effects from an extract of *Aplisina caissara*, a marine sponge fundament. *Clinical Pharmacology,* 52(5), 935-937.

Bergquist RP (1978) Sponges, *University of California Press,* Berkeley.

Blunt JW, Copp BR, Munro MH, Northcote PT and Prinsep MR (2005) Marine natural products, *Natural Product Reports,* 22, 15-61.

Blunt JW, Copp BR, Munro MH, Northcote PT and Prinsep MR (2006) Marine natural products, *Natural Product Reports,* 23, 26-78.

Chanas B, Pawlik JR, Lindel T and Fenical, W (1997) Chemical defence of the Caribbean sponge *Agelas clathrodes* (Schmidt). *Journal of Experimental Marine Biology and Ecology,* 208(1-2), 185-196.

Dayton PK, Robilliard GA, Paine RT and Dayton LB (1974) Biological accommodation in the benthic community at McMurdo Sound, Antarctic. *Ecological Monographs,* 44, 105-128.

Harborne JB (1998) Phytochemical methods. London: *Chapman and Hall*, 117–119.

Kanagasabhapathy M, Nagata K, Fujita Y, Tamura T, Okamura H and Nagata S (2004) Antibacterial activity of the marine sponge *Psammaplysilla purpurea*: Importance of its surface-associated bacteria. Proceedings of Oceans. *Techno-Ocean,* 4, 1323-1329.

Mokashe SS, Garg A, Anil AC and Wagh AB (1994) Growth inhibition of periphytic diatoms by

methanol extract of sponge and holothurians. *Indian Journal of Marine Sciences,* 23, 57–58.

National Committee for Clinical Laboratory Standerds (1990) Performance standards for antimicrobial disk susceptibility tests. *Approved Standard* M2-M4.

National Committee for Clinical Labotatory Standards (1990) Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. *Approved Standard* M7-M2.

Nigrelli, (1959) Ecology of toxicity in marine sponges. *Journal of Marine Biology.* 1432 – 1793.

Parulekar AH and Shirvoikar P (1991) Bioactivity and chemical ecology of some intertidal animals, pp. 29–35, *in* Bioactive Compounds from Marine Organisms. M. F. Thompson, R. Sarojani, R. Nagabhushanam (eds.). O*xford & IBH Publishing,* New Delhi.

Radjasa OK, Kencana DS, Sabdono A, Hutagalung RA and Lestari ES (2007) Antibacterial activity of marine bacteria associated with sponge Aaptos sp. against Multi drug resistant (MDR) strains. *Journal Matematika dan Sains,* 12, 147-152.

Reiswig HM (1973) Population dynamics of three Jamaician Demospongiae. *Bulletin of Marine Science,* 23, 191-226.

Sakemi S, Ichiba T, Kohmoto S and Saucy G (1988) Isolation and structure elucidation of onnamide A a new bioactive metabolite of a marine sponge *Theonella* sp. *Journal of American Chemical Society,* 110, 4851- 4853.

Selvin J and Lipton AP (2004) Antimicrobial activities of marine sponges collected off the coast of Santa Catarina – Biopotentials of secondary metabolites. *Hydrobiologia,* 513, 231-238.

Subugade S, Mokashe SS, Gupta SG, Lakwal VR and Kharate DS (2015) Antimicrobial potential of the marine intertidal sponge, *Ircinia fusca* from west coast of India. *Indian Journal of Pharmacology and Biological. Research,* 3(3), 12-14

Meesala S, Singh R and Watve MG (2017) Anti-*Mycobacterium* Metabolite from Marine Sponge. *Iricinia fusca. British Journal of Pharmaceutical Research, 15(4), 1-6.*

Sujatha S, Prakash GV and Jobi MD (2014) Marine sponge and its potential bioactive spectrum against CSF affected bacterial organisms. *The Pharma Innovation Journal* 3(6), 81-86.

Targett NM and Schmahl GP (1984) Chemical ecology and distribution of sponges in the Salt River Canyon, St Croix, U.S.V.I. NOAA Technical Memorandum OAR NURP-1, *Roxkville,* MD, 361-369.

Thakur NL and Anil AC (2000) Antibacterial activity of the sponge Ircinia ramose: importance of its surface- associated bacteria. *Journal of Chemical Ecology,* 26, 57-71.

Wenner EL, Knott DM, Van Dolah RF and Burrell VG (1983) Invertebrates communities associated with hard bottom habitats in south Atlantic Bight. *Estuariane, Coastal and Shelf Science,* 17, 143-158.