Anti-pathogenic efficacy of Indian edible macrofungi *Dacryopinax spathularia* (Schwein) and *Schizophyllum commune* (Fries) against some human pathogenic bacteriae

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***Abstract*:** In the present study the two edible macrofungi *Dacryopinax spathularia* and *Schizophyllum commune* were subjected to ethanolic extraction and the inhibitory impact of the extracts was tested by Agar diffusion method and Broth dilution method against five human pathogenic bacteria, *Viz. Pseudomonas aeruginosa* (MTCC 7837), *Staphylococcus aureus* (MTCC 3160), *Proteus mirabilis* (MTCC1429), *Bacillus subtilis* (MTCC736) and *Salmonella typhi* (MTCC3216) which are known to cause several diseases and infections like typhoid, urinary tract infection, pulmonary tract infection, pneumonia, staphylococcal scalded skin syndrome (SSSS). The Mycochemical constituents of both the macrofungal extracts include compounds with antibacterial properties like Phenols, Alkaloids, Saponins, Tannins, Flavonoids etc. The *Schizophyllum commune* extract show higher ZOI (Zone of inhibition) of 9 mm at 1000 microgm concentration against *P.mirabilis*, whereas *D. spathularia* extract shows higher ZOI of 7mm against *S.aureus* at 1000 microgram concentration. The *Dacryopinax spathularia* extract shows higher MIC (Minimum inhibitory concentration) 4 microgram/ml against *S.typhi*, whereas *S.commune* extract shows higher MIC 16 microgram/ml against *P. aeruginosa*. In broth dilution method both extracts achieved 100% inhibition.

IndexTerms: **Macrofungi; Mycochemical; antibacterial; Zone of inhibition; brothdilution**.

1. Introduction

Infectious diseases are the major cause of death across the world which may be caused by bacteria, virus, fungi, pathogenic protozoa or other pathogenic agents. During past few decades the pathogenic bacteria have emerged with drug resistance due to irrational use of antibiotics thereby decreasing the cure rate of infectious diseases which is an alarming situation and therefore the development of new potent antimicrobial agents is attaining more significance (Robin et al., 1998; Odonkor and Addo, 2011). Besides the efforts to develop synthetic antibacterial drugs, the use of herbs or crude extract having bioactive compounds derived from natural sources such as plants, animals, fungi etc. as antibacterial agents, is also gaining much attention (Bala et al., 2011; Genilloud, 2012). Fungi have been used as a potential source of medicinal agent since the first discovery of antibiotic penicillin from *Penicillium chrysogenum* (Hawksworth, 2001; Dutta and Acharya, 2014). In India a rich wealth of medicinal plants and mushrooms is present which are being used as folk medicines from centuries.

Macrofungi are commonly called as Mushrooms belonging to two major groups Ascomycota and Basidiomycota, which represents various gilled fungi, with or without stems, with fleshy fruiting bodies in some fungi or with woody or leathery fruiting bodies in others. The mushrooms or macrofungi are known to be rich sources of various bioactive substances like anti-bacterial, anti-fungal, anti-viral, anti-parasitic, anti-oxidant, anti-inflammatory, anti-proliferative, anti-cancer, anti-tumour, cytotoxic, anti-HIV, hypo-cholesterolemic, anti-diabetic, anti-coagulant, hepato-protective compounds, among others (Wasser and Weis, 1999). The therapeutic efficacy of macrofungi have also been extensively reviewed by Lindequist et

al (2005) and the antibacterial activity has been found in several macrofungal species by many workers (Efremenkova et al. 2003; Dugler et al., 2004a, 2004b). However, a large proportion (approximately 85-90%) of the total number of macrofungal species have not been described (Hawksworth, 2012) and hence there is a large proportion of macrofungal species whose medicinal and nutritional properties are yet to be identified. *Dacryopinax spathularia* (Schwein) and *Schizophyllum commune* (Fries) are the two edible macrofungi belonging to group Basediomycota and have been used traditionally for the treatment of various diseases and disorders such as antiviral, antitumour, antibacterial, and immunomodulating, anti-inflammatory , anti-diabetic, hepatoprotective activities (Mitko et al., 2008; Adebayo *et al*., 2012). Kumar *et al* (2018) have reported the mycochemical composition of the two edible macrofungi *Dacryopinax spathularia* and *Schizophyllum commune* and found that both the macrofungi contain potent mycochemicals like tannins, saponins, alkaloids, flavonoids, phenolics etc. in significant quantities. Further, the present study has been undertaken to investigate the antibacterial efficacy of *D. spathularia* and *S. commune* extracts against five pathogens *Viz. Psedomonas aeruginosa, Staphylococcus aureus, Proteus mirabilis, Bacillus subtilis* and *Salmonella typhi*.

2. Materials and methods

2.1 Collection of Macrofungi

Fresh fruiting bodies of *D. spathularia* and *S. commune* were collected from different sites of three National Parks (Orang National Park, Kaziranga National Park and Manas National Park) of Assam which were identified in laboratory of Department of Botany, Gauhati University, Guwahati and then they were carried to Department of Zoology, Ranchi University, Ranchi for analysis of their mycochemical constituents and determination of their pharmacological properties.

2.2 Extract Preparation

Extraction has been done using Soxhlet following standard extraction method. The fresh fungi have been washed, disinfected by treating with $HgCl_2$ and then washed repeatedly. After proper washing the fungi were dried under shade at room temperature for six to seven days. Then the fully dried fungi were powdered and sieved (Kumar et al., 2013). The fine powder of the fungi was subjected to extraction by soxhlet using distilled water for aqueous extract and ethanol for ethanolic extract. The obtained extract was filtered, concentrated and then dried at 45ºC in rotary flash evaporator for proper dehydration. The dried extract has been stored in air tight containers at room temperature for further experimental uses (Dandapat et al., 2014).

2.3 Mycochemical analysis

Qualitative analysis has been done to confirm the presence of potent mycochemicals like tannins, saponins, alkaloids, flavonoids, Phenols etc. in both the extracts prepared from the experimental macrofungi *D. Spathularia* and *S. Commune* (Table 1) (Gupta, 2003; Sofowara, 2008; Gupta et al., 2013; Ismail et al., 2017).

2.4 Antibacterial efficacy

The antibacterial efficacy of both the experimental macrofungi has been screened against five pathogenic bacteriae by agar plate diffusion method and broth dilution method.

2.4.1 Agar plate diffusion method

Following Threlfall et al., (1999), the stock cultures of bacteria were inoculated in broth media for revival and then grown at 37ºC for 18 hrs. The broth media were prepared on agar plates followed by making wells in them. Each plate was inoculated with 18 hrs. old cultures (100 µl, 10^{-4} cfu) and spread evenly on the plate. After 20 min, the wells were filled with different concentrations of extracts. The control wells with Ciprofloxacin were also prepared. All the plates were incubated at 37ºC for 24 h and the diameter of inhibition zone were measured.

2.4.2 Broth dilution method

As proposed by Walker (2000), the stock cultures of bacteria were revived by inoculating in broth

media and grown at 37ºC for 18 hrs. The tubes containing above media were prepared, sterilised in the autoclave and respective concentrations of the samples were added. Each tube was inoculated with 18 hrs. old cultures (100 µl, 10^4 cfu). A control tube with inoculums and without any sample was prepared along with a sterile media tube as blank. All the tubes were incubated at 37ºC on a shaker with 140 rpm for 24 h and the growth was measured at 660 nm. The % of inhibition was calculated by using the formula below

$$\% \text{ Inhbition} = 100 - \left[\frac{\text{OD of culture with sample (Test)}}{\text{OD of culture without sample (Control)}} \text{ X } 100 \right]$$

3. Results and discussion

The present work clearly reveals that both the experimental macrofungal extracts acheived 100% inhibition against all the tested pathogenic bacteriae at different concentrations (Figure 1 & 2). The mycochemical screening confirms the presence of potent chemical constituents like tannins, saponins, flavonoids, alkaloids, phenolics etc. in extracts of both macrofungi studied (Table 1),among which several mycochemicals have been reported to have the antibacterial properties. Kennedy and Wightman (2011) have reported that Tannins, alkaloids, saponins, flavonoids have growth inhibitory impact on *S. typhi*. Middleton and Kandaswamy (1994) reported that Flavonoids can inhibit several enzymes, chelate certain metal cations, affect protein phosphorylation and have variety of effects on membrane -linked processes. Flavonoids may inhibit the DNA gyrase enzyme activity, affects the energy metabolism and cytoplasmic membrane function (Cushine et al., 2006) . Isaac and Chinwe (2001) reported that alkaloids are responsible for the antibacterial activity. Shachi et al., (2011) reported that alkaloids possess inhibitory effect on bacterial growth. Cushine et al., (2014) reported that alkaloids can disrupt the bacterial fimbrae and other adhesins, inhibits the bacterial defence mechanisms against host cell, affects bacterial cell wall structure, inhibits the bacterial secretion systems and can produce other antibacterial effects. Augustin Scalbert (1991) reported that tannins possess antibacterial activities.

The present work reveals that both the macrofungal extracts studied have significant antibacterial activity, which can be attributed to the potent mycochemical constituent compounds like tannins, saponins, flavonoids, alkaloids etc. present in them. The results of the present work reveals that in agar plate diffusion method the *D. spathularia* extract shows maximum ZOI of 7 mm at 1000 microgram concentration against *S. Aureus*, whereas *S. commune* extract shows maximum ZOI of 9 mm at 1000 microgram concentration against *P. Mirabilis* (Table 2). In broth dilution method, the results showed that the *D. Spathularia* extract is having 100% inhibition impact ranging from 1 mg/ml against S. aureus, P. aeruginosa, P. Mirabilis and B. subtilis to 4 mg/ml against S. typhi, whereas the *S. Commune* extract is having 100% inhibition impact ranging from 1 mg/ml against S. Aureus and B. Subtilis, 2 mg/ml against S. Typhi, 4 mg/ml against P. Mirabilis and 16 mg/ml against P. Aeruginosa. The above result clearly indicates that the two experimental macrofungi have significant inhibitory impact against all the five tested bacteria, showing 100% inhibitions against all the tested bacteria.

4. Conclusion

Based on the results of the present work, it can be concluded that the two macrofungi studied have the potent mycochemical constituents having anti-bacterial activity, which are responsible for the significant anti-bacterial imapact of both macrofungal extracts. The present results may open the way to use the above two macrofungi as potent antimicrobial dietary sources or to the development of new potent antibacterial drugs.

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Figure and Table legends

Figure 1 : Showing % inhibition of *D. spathularia* extract against *S. aureus* (2), *P. aeruginosa* (3), *P. mirabilis* (4), *S. typhi* (5) and *B. Subtilis* (6).

Figure 2 : Showing % inhibition of *S. commune* extract against *S. aureus* (2), *P. aeruginosa* (3), *P. mirabilis* (4), *S. typhi* (5) and *B. Subtilis* (6)
Figure 3 : Zone of inhibition of extract of *S. commune* against different bacteriae
Figure 4: Zone of inhibition of antibiotic Ciprofloxacin against different bacteriae
Figure 5: Zone of inhibition of extract of *D. spathularia* against different bacteriae
Table 1 : Qualitative analysis of mycochemicals in macrofungal extracts of and *S.commune*
Table 2 : The diameter of inhibition zones (in mm) against extracts of two experimental macrofungi and anibiotic Ciprofloxacin in agar plate diffusion method

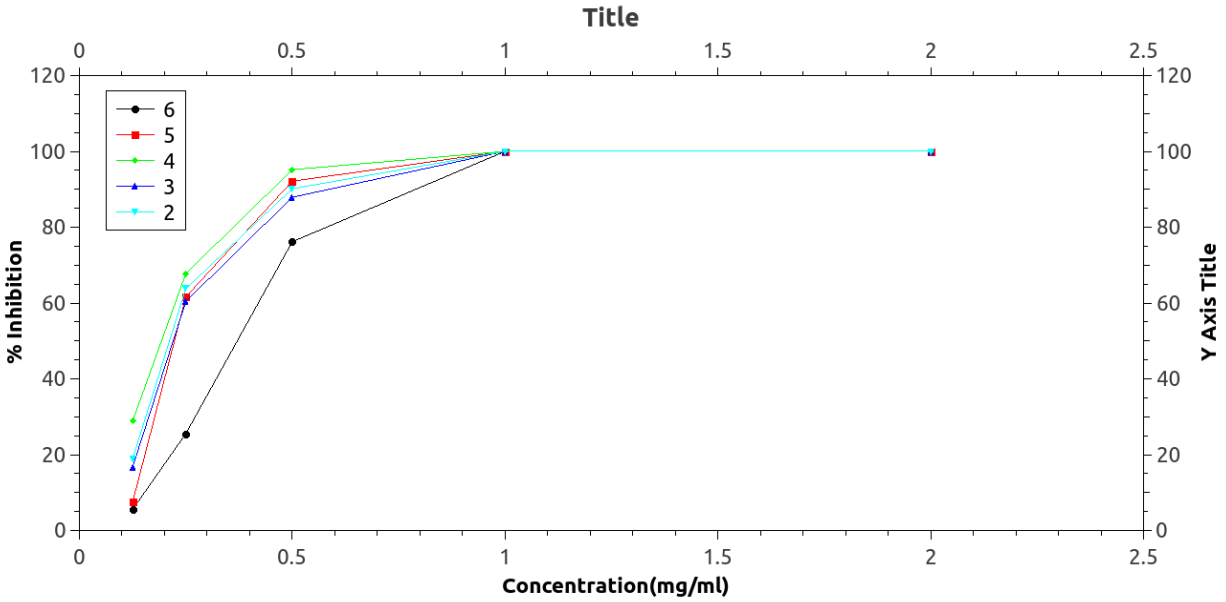

Figure 1 : Showing % inhibition of *D. spathularia* extract against *S. aureus* (2), *P. aeruginosa* (3), *P. mirabilis* (4), *S. typhi* (5) and B. Subtilis (6).

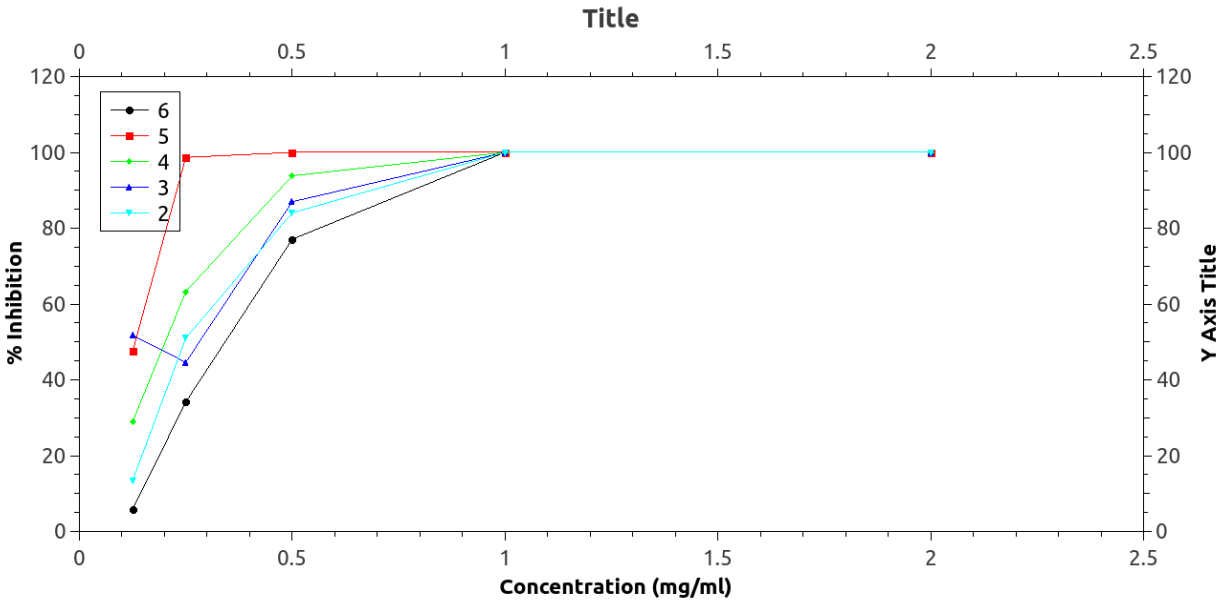

Figure 2 : Showing % inhibition of *S. commune* extract against *S. aureus* (2), *P. aeruginosa* (3), *P. mirabilis*

(4), *S. typhi* (5) and B. Subtilis (6)

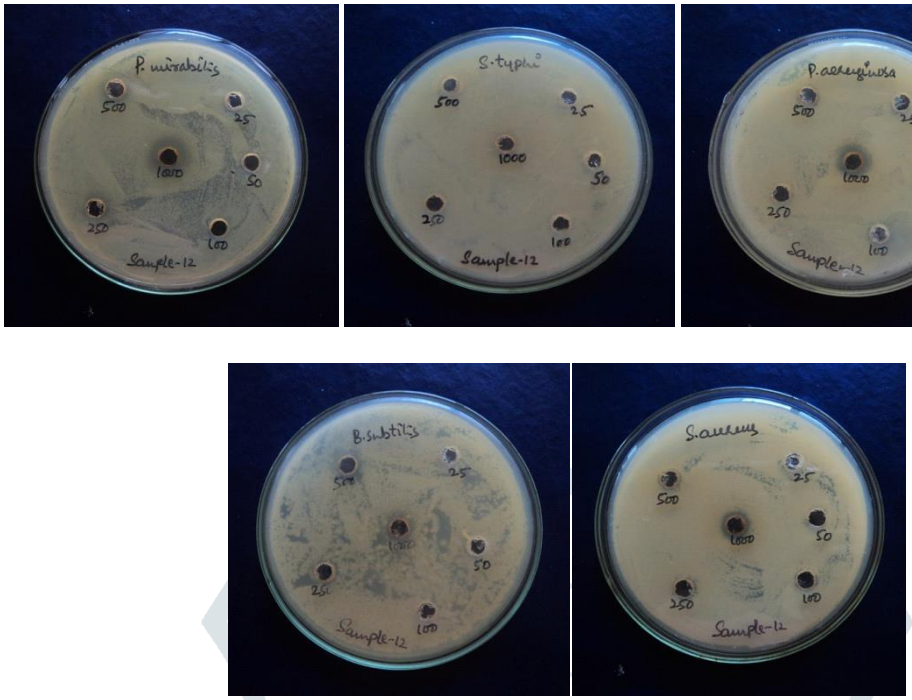

Fig. 3: Zone of inhibition of extract of *S. commune* against different bacteriae

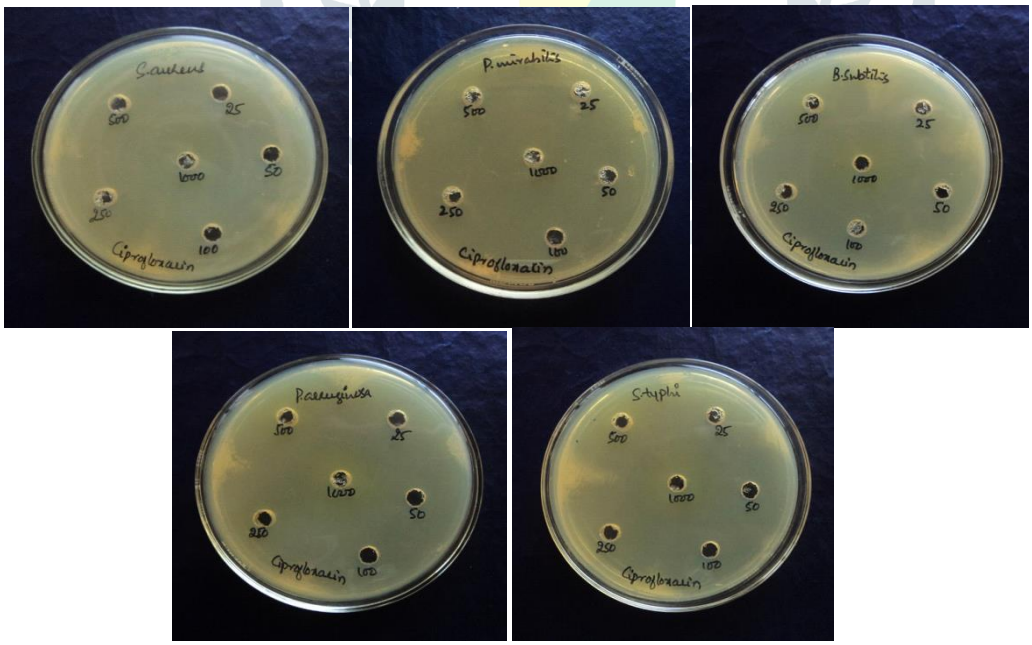

Fig.4: Zone of inhibition of antibiotic Ciprofloxacin against different bacteriae

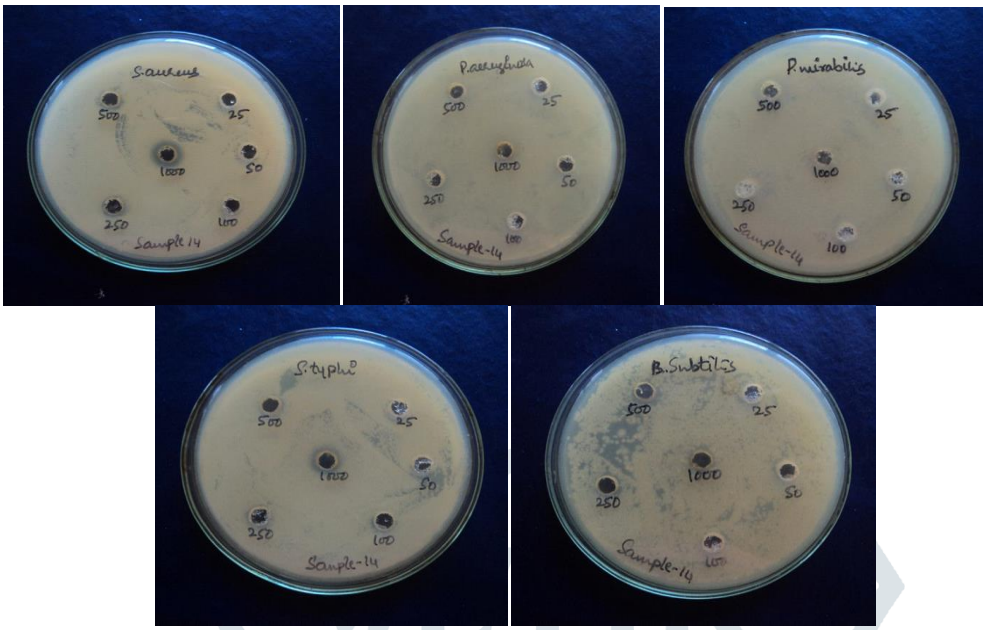

Fig 5 : Zone of inhibition of extract of *D. spathularia* against different bacteriae

Table1 : Qualitative analysis of mycochemicals in macrofungal extracts of D. Spathularia and S.commune

Tests and reagents	**Observation**	**Inference**	**D. spathularia**	**S. commune**
1ml sample + 3ml Drangendroff reagent, mixed well, boiled for 5 minutes	Dark brown colour or orange colour	Alkaloids present	+	+
Mayer's test :- 1ml sample + 1 ml mayer's reagent, mixed carefully	White or pale yellow colour	Alkaloids present	+	+
1 ml sample + 2 ml sulphuric acid added, mixed well	Yellow colour formation	Flavonoids present	+	+
Shinoda test :- 2 ml sample + 5 ml of 95% ethanol + few drops conc. Hcl + 0.5 gm magnesium turnings	Yellow colour formation	Flavonoids present	+	+
1 ml sample + 2 ml 5% $FeCl_3$ solution	Deep blue black colour appeared	Tannins present	+	+
1 ml sample + 2 ml dil. HNO_3 solution	Reddish colour appeared	Tannins present	+	+
Few drops of sample heated with alcoholic KOH, then boiled for 1 minute and cooled. Then the mixture is acidified with 1 ml conc. Hcl. A portion of mixture was treated with 10 ml water and then 5% NaOH added dropwise	Clear soap was observed while shaking	Saponins present	+	+

2ml sample + 2 ml aquous Ferric Chloride	Blue colour appeared	Phenols present	+	+

Table 2 : The diameter of inhibition zones (in mm) against extracts of two experimental macrofungi and anibiotic Ciprofloxacin in agar plate diffusion method

Schizophyllum commune

Organism	**25 µg**	**50 µg**	**100 µg**	**250 µg**	**500 µg**	**1000 µg**	**MIC µg**
S. aureus	0	0	0	0	0	5	1000
P. aeruginosa	0	0	0	0	0	7	1000
P. mirabilis	0	0	0	0	0	9	1000
S.typhi	0	0	0	0	0	0	NF
B. subtilis	0	0	0	0	0	2	1000

Dacryopinax spathularia

Organism	**25 µg**	**50 µg**	**100 µg**	**250 µg**	**500 µg**	**1000 µg**	**MIC µg**
S. aureus	0	0	0	0	0	7	1000
P. aeruginosa	0	0	0	0	0	0	NF
P. mirabilis	0	0	0	0	0	0	NF
S.typhi	0	0	0	0	0	3	1000
B. subtilis	0	0	0	0	0	2	1000

Ciprofloxacin

Organism	**25 µg**	**50 µg**	**100 µg**	**250 µg**	**500 µg**	**1000 µg**	**MIC µg**
S. aureus	25	28	31	34	36	*	25
P. aeruginosa	30	32	34	35	38	*	25
P. mirabilis	*	*	*	*	*	*	25
S.typhi	27	31	35	38	40	*	25
B. subtilis	20	24	27	30	36	*	25

Note: NF- MIC not found in the concentrations screened

*Ciprofloxacin inhibition zones could not be measured due to merging of zones because of high activity of antibiotic

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