

lipophilic drug towards hydrophilic polymer, as it has been proved for some hydrophilic drugs like doxorubicin with higher affinity towards natural hydrophilic polymers.

In the *in vitro* release study of both ethyl cellulose and methyl cellulose nanospheres, the initial burst is considered to be due to the release of the drug located on the particle surface, and the second part of the release profile could be due to the drug diffusing out of the polymer particles and the breakdown of polymeric material. The same phenomenon has been observed by Gurny *et al.* for PLA nanospheres containing testosterone.

This study has shown that distinct and discrete nanospheres containing etoposide can be prepared from ethylcellulose and methylcellulose and the drug entrapment efficiency of methylcellulose is relatively more than the ethylcellulose. The *in vitro* release profile of drug from both the polymers indicates their potentiality and suit-

ability for targeted distribution of anti-cancer drugs. However, a thorough stability analysis and *in vivo* drug distribution of these nanospheres are yet to be established.

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Antibacterial and Antifungal Activities of Marine Sponges

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Free fatty acid mixtures obtained from the hydrolysis of lipids isolated from seventeen marine sponges collected from the Orissa coast were screened against selected human pathogenic bacteria and fungi. The fatty acid components of two species, *Plakina monolopha* and *Callyspongia sp.* showed antibacterial activity against *Vibrio cholerae* and *Pseudomonas aeruginosa* while the fatty acid components of *Callyspongia sp.* showed antifungal activity against *Candida albicans*. These components have not shown any inhibitory activity against the useful human intestinal bacterium *Bacillus iaterosporus*.

Marine sponges have been reported¹⁻⁹ earlier as a source of lipids, glycolipids, phospholipids and corresponding long chain saturated and unsaturated fatty acids besides other novel compounds. Fatty acids obtained from marine algae have shown antimicrobial activity^{9,10} and fatty acids from Caribbean sponges have been

reported earlier to possess antifungal activity¹¹. Hence, an attempt is made to investigate the antibacterial and the antifungal activities of free fatty acid components of 17 marine sponges collected from the Orissa coast¹² by SCUBA divers from a depth of 25-30 m. Two out of 17 sponges, *Plakina monolopha* (family: Halinidae) and *Callyspongia sp.* (family: Callyspongiidae) showed activity and the results are presented in this paper (Table-1).

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Fatty acids from other fifteen sponges; *Acanthella cavernosa* (Dendy), *Acanthella elongata* (Dendy), *Spirastrella vagabunda* Ridley, *Hyatella cribriformis* (Hyatt), *Heteronema erecta* Keller, *Spirastrella inconstans* (Dendy), *Callyspongia fibrosa* (Ridley and Dendy), *Plakortis simplex* (Schulze), *Raspailia* sp., *Epipolasis* sp., *Oceanopia* sp., *Azorica pfeifferae* Carter, *Petrosia testudinaria* (Lamarck), *Psammaplysilla purpurea* (Carter) and *Phakellia dendyi* Bergquist were found inactive. The individual sponge samples were thoroughly washed, cut, air-dried, homogenized and repeatedly extracted with chloroform-methanol (2:1 v/v), to isolate lipids¹³. Crude lipids were purified¹⁴ and saponified to obtain the free fatty acids, which were then extracted with diethyl ether, and the solvent was removed under reduced pressure. These were stored in a refrigerator. Fatty acid components of each species so obtained were tested separately for antibacterial activity against *Escherichia coli*, *Vibrio cholerae*, *Pseudomonas aeruginosa* and for antifungal activity against *Candida albicans*. *Bacillus laterosporus* is a useful and non-lactic acid producing bacterium found in the human intestines. It helps in rapid colonization of any beneficial flora. The fatty acid components were also tested against these bacteria.

For the antibiotic activity assays, the *in vitro* filter paper disc diffusion method¹⁵ was adopted. Nutrient agar and Sabouraud's Dextrose agar were used to culture the bacteria and fungi respectively. Peptone water (1%) was used for fresh culture of all the bacteria and fungi and were maintained by periodic sub culturing in fresh Nutrient agar and Sabouraud's Dextrose medium.

Plates for Nutrient agar and Sabouraud's Dextrose medium were prepared with the inocula by adding 1 ml of diluted culture of the test organism. The fatty acid components obtained from both the species were liquid in nature. For each species different concentrations of fatty acid components (v/v) viz., 10 µl, 5 µl, 2.5 µl and 1.3 µl dissolved each in 10 µl chloroform were prepared separately and each was poured on sterile filter paper disc (5 mm dia.) using a micropipette. The discs were then allowed to dry in inert nitrogen atmosphere and aseptically transferred to agar plates already seeded with the test organisms. Solvent control and reference standard antibiotic (10 µg) discs of gentamicin and ciprofloxacin for bacteria and amphotericin-B for fungus were placed on the respective seeded medium. For assaying, antibacterial activity plates were incubated at 37° for 24 h

whereas for antifungal activity they were incubated at 30° for 72 h. The diameter of the zone of inhibition (mm) was measured as an average of maximum dimensions of zones around the discs. All the experiments were carried out in triplicate and the average values were recorded in Table-1.

From the table-1 it is evident that fatty acid components of both the species are endowed with marked antibacterial activity against *V. cholerae* and *P. aeruginosa* even in gradually decreased concentrations. The fatty acid components of *Plakina monolopha* have shown more antibacterial activity against *V. cholerae* than that shown by the fatty acid components of *Callyspongia* sp. even at lower concentration. Further, the results were compared with standard antibiotic discs of gentamicin and ciprofloxacin. The fatty acid components of *Plakina monolopha* showed the inhibitory activity in 10 µl/disc concentration against *V. cholerae* equal to that of the standard antibiotic gentamicin at 10 µg/disc. Also, test components of both the species showed inhibitory activity against *P. aeruginosa* almost equal to that of gentamicin, but lower than that of ciprofloxacin.

The data on antifungal activity revealed that fatty acid components of only *Callyspongia* sp. showed marked antifungal activity against *C. albicans* but less than that exhibited by standard antifungal antibiotic amphotericin-B. No inhibitory activity was observed for the fatty acid components of both the species against *E. coli*. It is note worthy that the fatty acid components of these sponges did not show any inhibitory activity against the useful human intestinal bacterium *B. laterosporus*.

From the above findings it can be noted that the unknown fatty acid components of these two marine sponges may be useful against microbial infections caused by human pathogenic organisms *V. cholerae*, *P. aeruginosa* and *C. albicans* and this needs further investigation.

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TABLE 1 : *IN VITRO* ANTIBACTERIAL AND ANTIFUNGAL ACTIVITY OF FATTY ACID COMPONENTS OF *PLAKINA MONOLOPHA* AND *CALLYSPONGIA SP.*

Fatty acid component/ Standard	Amount/ disc (μ l)	Diameter of Zone of Inhibition in mm*				
		V. <i>cholerae</i>	E. <i>coli</i>	P. <i>aeruginosa</i>	B. <i>laterosporus</i>	C <i>albicans</i>
1. <i>Plakina monolopha</i>	10.0	23.0	NI	16.5	NI	NI
	5.0	19.0	NI	13.0	NI	NI
	2.5	15.0	NI	10.0	NI	NI
	1.3	11.5	NI	7.0	NI	NI
2. <i>Callyspongia sp.</i>	10.0	20.0	NI	16.0	NI	17.5
	5.0	14.5	NI	13.0	NI	15.5
	2.5	11.0	NI	9.5	NI	13.0
	1.3	9.0	NI	7.0	NI	11.0
Standard	(μ g)					
Gentamicin	10.0	23.0	19.0	16.0	---	---
Ciprofloxacin	10.0	26.0	22.0	23.5	---	---
Amphotericin-B	10.0	---	---	---	---	31.0
Vehicle (Chloroform)		Nil	Nil	Nil	Nil	Nil

*Including the diameter of the disc, 5 mm, NI - No Inhibition; Values are average of three determinations

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