Table 1. Antifouling activity of secondary metabolites isolated from *H. scabra*

Concentration of extract (mg/ml)	Fouling (%)	Regaining (%)	Behavioural changes of snails observed during exposure and after in fresh sea water
1.05	72 ± 9.8	98 ± 6.0	Moving with spread foot
2.1	14 ± 4.9	94 ± 9.16	Attached with spread foot; regained immediately
4.2	0	78 ± 5.74	Shrunken foot; regained after 5 min
6.5	0	10 ± 4.26	Shrunken foot; mortality

Values in mean ± SD; $n = 10$.

organisms require a suitable surface for their foot adherence. The attachment process involves recognition of the surface and production of biological adhesives that ensure attachment. Based on this principle, the present assay was developed, which have the following merits/advantages over the conventional assay technique:

- As the assayed snail is a common rock fouler, it could be easily collected from all over the coastal area.
- The test plates could be prepared with minimum quantity of test compound.
- The assay can be completed within an hour.
- Usually the snail finds a suitable place initially and spreads its foot for adherence. Therefore the rate of fouling could be easily monitored under a glass surface based on the reflex of foot adherence.
- After the experiment, the relative toxicity could also be estimated based on the regaining rate in fresh seawater.
- The mechanism of antifouling activity of the compounds on experimental *P. vulgata* may be due to the failure of recognition of suitable surface (due to sensory impairments) or inhibition of production of its biological adhesive required for attachment.

Considering the above merits over the conventional 'foot stimulating' assay, it could form a reliable alternate method for assessing the potent antifouling activity and bioassay-guided purification processes.

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Antibacterial activity of *Curcuma longa* rhizome extract on pathogenic bacteria

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Curcuma longa **rhizome extracts were evaluated for antibacterial activity against pathogenic strains of Gram-positive (*Staphylococcus aureus*, *Staphylococcus epidermidis*) and Gram-negative (*Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella typhimurium*) bacteria. Essential oil was found to be most active and its activity was compared to standard antibiotics gentamycin, ampicillin, doxycycline and erythromycin in these strains. Only the clinical isolate of *S. aureus* showed more sensitivity towards essential oil fraction than the standard strain. The use of essential oil from turmeric as a potential antiseptic in prevention and treatment of antibacterial infections has been suggested.**

CURCUMA longa, commonly known as 'turmeric', is widely used as a spice and colouring agent, and is known

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for its medicinal properties[1]. Various sesquiterpenes[2] and curcuminoids[3] have been isolated from the rhizome of *C. longa*, attributing a wide array of biological activities such as antioxidant[4], anti-inflammatory[5], wound healing[6], anticancer and antiproliferative[7,8], antifungal[9] and antibacterial[10] activity.

The development of bacterial resistance to presently available antibiotics has necessitated the search for new antibacterial agents[11]. The Gram-positive bacteria such as *Staphylococcus aureus* are mainly responsible for post-operative wound infection, toxic shock syndrome and food poisoning. The Gram-negative bacterium such as *E. coli* is present in human intestine and causes lower urinary tract infection, coleocystis or septicemia. The Gram-positive and Gram-negative bacteria can be inhibited by antibiotics, either by blocking the protein synthesis or peptidoglycan synthesis in bacterial cell wall. The antibacterial activity of turmeric was reported as early as 1956 (ref. 12). We report here the effect of various extracts of turmeric on pathogenic strains of Gram-positive bacteria, *S. aureus* (standard reference (SR) ATCC 6571 and clinical isolate (CI)), and *Staphylococcus epidermidis* (WHO-6); and Gram-negative bacteria, *E. coli* (standard reference (SR) ATCC 10418 and clinical isolate (CI)), *Pseudomonas aeruginosa* (standard reference (SR) ATCC 10662 and clinical isolate (CI)), *Salmonella typhimurium* (lab. strain) by zone of inhibition assay[13] and their effect was compared to various antibiotics, viz. gentamycin, ampicillin, doxycycline and erythromycin, with different mechanisms of action on bacteria.

Fresh rhizomes of *C. longa* were obtained as a gift from National Bureau of Plant Genetic Resources, Regional Station, Trissur. The rhizomes (200 g) were ground finely in a mortar and pestle by adding little water and were subjected to steam distillation. The oily fraction (fraction-I, 0.763 g) was collected and the residue in water was filtered. The filtrate was evaporated under vacuum to give water extract (fraction-IV, 2.0 g). The residue was air-dried and left overnight in chloroform (200 ml), filtered and re-extracted twice with chloroform (2 × 100 ml). All the chloroform extracts were combined and solvent was evaporated to give chloroform extract (fraction-II, 1.0 g). The residue left after chloroform extract, was extracted with methanol to give the methanol extract (fraction-III, 3.8 g; Figure 1).

The Gram-positive (*S. aureus* SR ATCC 6571 and clinical isolate, and *S. epidermidis* (WHO-6) and Gram-negative (*E. coli* SR ATCC 10418 and clinical isolate, *P. aeruginosa* SR ATCC 10662 and clinical isolate and *S. typhimurium* lab strain) bacteria used as test organisms were obtained from All India Institute of Medical Sciences, New Delhi. Cultures of bacteria were grown on nutrient broth (Hi Media, Mumbai) at 37°C for 12–14 h and were maintained on nutrient agar slants (Hi Media, Mumbai) at 4°C. The extracts were dissolved in ethylene glycol, membrane-filter (0.47 μm) sterilized and tested for antibacterial activity using disc diffusion method. A concentration of 2000 μg/disc was chosen based on available literature[13,14]. Sterile 6-mm diameter filter-paper discs were impregnated with 2000 μg of the sterile test material, and placed onto nutrient agar surface spread with 0.1 ml of bacterial culture (ca. 3×10^8 cells/ml using McFarland's 1 as standard). The plates were incubated at 37°C for 12–14 h. The experiments were carried out in triplicate. The results (mean value $n = 3$) were recorded by measuring the zone of growth inhibition around the discs. The statistical analysis was carried out using Student's *t* test[15]. Control discs contained ethylene glycol only. For comparison, standard antibiotics gentamycin and ampicillin inhibiting bacterial cell wall biosynthesis, and doxycycline and erythromycin inhibiting bacterial protein synthesis were included in the assay.

The antibacterial spectra showing zone of inhibition in millimetres and as percentage (calculated by taking gentamycin as positive control with 100% inhibition) for Gram-positive and Gram-negative bacteria are shown in Tables 1 and 2 respectively. Various extracts (Figure 1) such as steam-distilled (fraction-I, essential oil), chloroform (fraction-II), methanol (fraction-III) and water (fraction-IV) were tested for antibacterial activity in *in vitro* systems. All the fractions were inactive against Gram-positive *S. aureus* (SR, ATCC 6571) and *S. epi-*

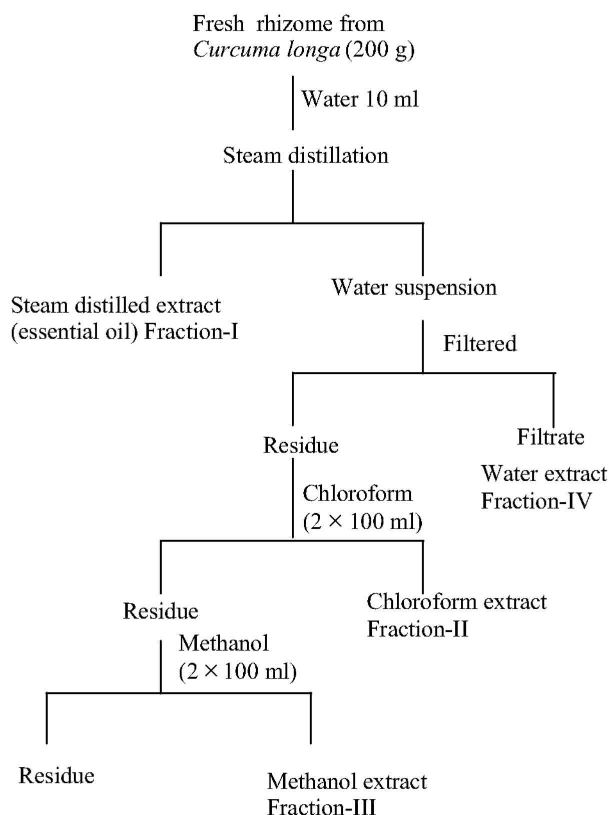

Figure 1. Preparation of extract.

Table 1. Zone of inhibition for various extracts from *Curcuma longa* compared to reference drugs: activity against Gram-positive bacteria

Microorganism →	*Staphylococcus aureus* SR Zone of inhibition		*Staphylococcus aureus* CI Zone of inhibition		*Staphylococcus epidermidis* Zone of inhibition	
Name of drug ↓	In mm Mean	As percentage$	In mm Mean	As percentage	In mm Mean	As percentage
Gentamycin 30 mcg	27.67 ± 1.47	100	10.33 ± 0.33	100	19.66 ± 0.87	100
Ampicillin 10 mcg	25.33 ± 0.88	89	6.00 ± 0.00	00	7.33 ± 0.32	10
Doxycycline 30 mcg	25.66 ± 0.66	90	7.66 ± 1.87	38	17.50 ± 0.86	84
Erythromycin 10 mcg	14.00 ± 1.35	37	7.66 ± 0.32	38	6.00 ± 0.00	00
Fraction-I	12.66 ± 0.88	31	16.66 ± 0.32	246	17.33 ± 0.87	83
Fraction-II	6.00 ± 0.00	00	6.00 ± 0.00	00	8.66 ± 0.32	19
Fraction-III	6.00 ± 0.00	00	6.00 ± 0.00	00	7.16 ± 0.16	08
Fraction-IV	9.33 ± 0.33	15	9.33 ± 0.66	77	7.50 ± 0.26	11
Ethylene glycol	6.00 ± 0.00	00	6.00 ± 0.00	00	6.00 ± 0.00	00

SR, Standard reference; Cl, Clinical isolates.
Mean, Mean value of diameter of inhibition zone with standard error.
As the diameter of paper disc used was 6 mm, 6 mm diameter included in the table is indicative of no activity.
$Percentage was calculated after subtracting disc diameter (6 mm) from all observations.

Table 2. Zone of inhibition for various extracts from *C. longa* compared to reference drugs: activity against Gram-negative bacteria

Microorganism →	*Escherichia coli* SR Zone of inhibition		*Escherichia coli* CI Zone of inhibition		*Salmonella typhimurium* Zone of inhibition		*Pseudomonas aeruginosa* SR Zone of inhibition		*Pseudomonas aeruginosa* CI Zone of inhibition	
Name of drug ↓	In mm Mean	As percentage	In mm Mean	As percentage	In mm Mean	As percentage	In mm Mean	As percentage	In mm Mean	As percentage
Gentamycin 30 mcg	24.00 ± 0.57	100	13.00 ± 0.57	100	25.16 ± 0.71	100	26.33 ± 0.87	100	21.33 ± 0.87	100
Ampicillin 10 mcg	16.00 ± 0.57	55	6.00 ± 0.32	00	6.66 ± 0.32	03	6.33 ± 0.32	24	6.00 ± 0.00	00
Doxycycline 30 mcg	20.33 ± 0.87	80	6.00 ± 0.00	00	6.00 ± 0.00	00	6.00 ± 0.00	00	6.00 ± 0.00	00
Erythromycin 10 mcg	15.66 ± 0.87	54	6.00 ± 0.00	00	6.00 ± 0.00	00	6.00 ± 0.00	00	6.00 ± 0.00	00
Fraction-I	8.66 ± 0.32	14	8.66 ± 0.00	38	12.66 ± 0.88	35	9.33 ± 0.32	16	8.66 ± 0.32	17
Fraction-II	6.00 ± 0.00	00	6.00 ± 0.00	00	6.00 ± 0.00	00	6.00 ± 0.00	00	6.00 ± 0.00	00
Fraction-III	6.00 ± 0.00	00	6.00 ± 0.00	00	6.00 ± 0.00	00	6.00 ± 0.00	00	6.00 ± 0.00	00
Fraction-IV	8.00 ± 0.57	11	7.00 ± 0.57	14	7.00 ± 0.00	05	7.00 ± 0.57	04	7.66 ± 0.32	04
Ethylene glycol	0.00 ± 0.00	00	0.00 ± 0.00	00	0.00 ± 0.00	00	0.00 ± 0.00	00	0.00 ± 0.00	00

Table 3. Minimum inhibitory concentration of fraction-I (essential oil) on Gram-positive bacteria with gentamycin as standard reference

Microorganism →	*Staphylococcus aureus* SR Zone of inhibition		*Staphylococcus aureus* CI Zone of inhibition		*Staphylococcus epidermidis* Zone of inhibition	
Name of drug ↓	In mm Mean	As percentage	In mm Mean	As percentage	In mm Mean	As percentage
Gentamycin 30 mcg	26.33 ± 0.74	100	9.67 ± 0.32	100	19.33 ± 0.66	100
Fraction-I	13.33 ± 0.32	36	16.33 ± 0.33	221	18.00 ± 0.58	90
1/10 dilution of fraction-I	11.66 ± 0.66	28	15.67 ± 0.33	207	16.33 ± 0.58	78
1/100 dilution of fraction-I	10.33 ± 0.32	21	12.67 ± 0.33	142	15.67 ± 0.33	73
Ethylene glycol	6.00 ± 0.00	00	6.00 ± 0.00	00	6.00 ± 0.00	00

dermidis (WHO 6) except fraction-I (essential oil). In *S. aureus* clinical isolate fraction-IV was also active, although fraction-I showed more activity than standard antibiotics (Table 1). Among Gram-negative bacteria, fraction-I displayed moderate activity against *E. coli* (CI) and *S. typhimurium* (lab strain), while all other extracts were inactive.

Therefore minimum inhibitory concentration (MIC) was studied only for essential oil fraction (fraction-I) and results were compared with standard antibiotics. It was

Table 4. Minimum inhibitory concentration of fraction-I (essential oil) on Gram-negative bacteria with gentamycin as standard reference

Microorganism →	*Escherichia coli* SR Zone of inhibition		*Escherichia coli* CI Zone of inhibition		*Salmonella typhimurium* Zone of inhibition		*Pseudomonas aeruginosa* SR Zone of inhibition		*Pseudomonas aeruginosa* CI Zone of inhibition	
Name of drug ↓	In mm Mean	As percentage	In mm Mean	As percentage	In mm Mean	As percentage	In mm Mean	As percentage	In mm Mean	As percentage
Gentamycin 30 mcg	24.33 ± 0.32	100	13.33 ± 0.32	100	24.50 ± 0.29	100	24.66 ± 0.87	100	21.33 ± 0.66	100
Fraction-I	9.33 ± 0.40	18	9.33 ± 0.32	45	11.67 ± 0.33	31	10.00 ± 0.58	22	9.00 ± 0.58	20
1/10 dilution of fraction-I	10.66 ± 0.33	25	8.00 ± 0.00	27	9.67 ± 0.33	20	8.66 ± 0.33	14	9.33 ± 0.32	22
1/100 dilution of fraction-I	8.00 ± 0.58	11	7.66 ± 0.33	23	6.00 ± 0.00	00	7.00 ± 0.00	05	8.33 ± 0.32	15
Ethylene glycol	6.00 ± 0.00	00	6.00 ± 0.00	00	6.00 ± 0.00	00	6.00 ± 0.00	00	6.00 ± 0.00	00

observed that dilution altered the activity gradually in Gram-negative bacteria to 80 and 60% against *S. aureus* (SR) at 1/10 and 1/100 dilutions respectively; however *S. aureus* (CI) did not show significant change (93%) at 1/10 dilution, although activity decreased to 64% at 1/100 dilution. In *S. epidermidis*, activity is decreased with 1/10 and 1/100 dilutions to 87 and 80% respectively (Table 3). The Gram-negative bacteria *E. coli* (SR), *P. aeruginosa* (SR) and *S. typhimurium* (lab strain) showed decrease in activity with dilution. *S. typhimurium* showed no activity below 200 μg/disc concentration (1/10 dilution). The activity of fraction-I against *E. coli* (SR) and *P. aeruginosa* (CI) was not affected at 1/10 dilution (Table 4).

Furthermore, essential oil fraction is more effective against Gram-positive compared to Gram-negative strains. *S. aureus* (CI) and *S. epidermidis* have shown 246 and 83% inhibition respectively, compared to Gram-negative strains of *E. coli* (CI), *P. aeruginosa* (CI), *S. typhimurium* showing inhibition of 38, 17, 35% respectively. However, no significant activity ($P > 0.1$) is shown in standard reference strains of both Gram-negative and Gram-negative bacteria. Fraction-I shows comparable activity to the standard antibiotics. It has shown highest inhibition in Gram-positive *S. aureus* (CI, 246%) and comparable activity in *S. epidermidis* (83%) compared to standard antibiotics ampicillin, doxycycline, erythromycin and gentamycin (Table 1). In Gram-negative bacteria all antibiotics were inactive, except gentamycin, while fraction-I showed significant ($P < 0.05$) activity (38 and 35%) against *E. coli* (CI) and *S. typhimurium* (lab. strain) as compared to gentamycin (Table 2). The result is encouraging, as all other antibiotics were inactive against this strain. The present study suggests that essential oil fraction from turmeric possesses significant ($P < 0.001$) antibacterial activity at very low concentration (20 μg/disc) on pathogenic Gram-positive *S. aureus* (CI) bacteria. The clinical isolates which are actually responsible for the invasion and infection and have developed resistance to the standard antibiotics, were sensitive to this fraction.

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