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Evaluation of antimicrobial activity of *Curcuma longa* rhizome extract against *Staphylococcus aureus*☆

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ABSTRACT

The in vitro antimicrobial activity of different fractions obtained from rhizome of *Curcuma longa* was investigated against standard strain and clinical isolates of *Staphylococcus aureus*. The clinical isolates were found more sensitive for different fractions, than the standard strain of *S. aureus*. Scanning electron microscopic observations revealed that test pathogen treated with *C. longa* extract showed morphological deformity, with partial lack of the cytoplasmic membrane, which leads to cell disruption The ability of rhizome of *C. longa* extracts to inhibit the growth of test pathogen is an indication of its broad spectrum antimicrobial potential which may be employed in the management of microbial infections.

1. Introduction

Nosocomial infections are hospital acquired infections. Historically, staphylococci, pseudomonads, and *Escherichia coli* have been the nosocomial infection troika. It is estimated that in United States in 1995, nosocomial infections cost $4.5 billion and contributed to more than 88,000 deaths—one death every 6 min [24]. In the study from 1990 to 1996, the three most common gram-positive pathogens—*Staphylococcus aureus*, coagulase-negative staphylococci, and enterococci— accounted for 34% of nosocomial infections, and the four most common gram-negative pathogens—*E. coli*, *Pseudomonas aeruginosa*, *Enterobacter* spp., and *Klebsiella pneumoniae*—accounted for 32% of nosocomial infections [21].

Major force involved in nosocomial infections is indiscriminate antimicrobial use in hospitals and long-term care facilities. Widespread use of cephalosporin antibiotics is often cited as a cause of the emergence of MRSA, which became a major nosocomial threat. *S. aureus* has shown to exhibit resistance to wide range of commonly available antibiotics especially penicillin [14,16]. Methicillin–resistant *S. aureus* (MRSA) is a major nosocomial pathogen [25]. According to a study, the MRSA prevalence has increased from 12% in 1992 to 80.83% in 1999 [30].

In addition to this problem, antibiotics are sometimes associated with adverse effects on the host including hypersensitivity, immune-suppression and allergic reactions [1]. Because of the side effects and the resistance that pathogenic microorganisms build against antibiotics, recently much attention has been paid to extracts and biologically active compounds isolated from plant species used in herbal medicine [13].

However, the potential of higher plants as sources for new drugs is still largely unexplored. India is the largest producer of medicinal herbs and appropriately called the botanical garden of the world [2]. Coincidentally, the last decade has also witnessed increasing intensive studies on extracts and biologically active compounds isolated from plant species used for natural therapies or herbal medicine [23].

Turmeric (*Curcuma longa* L.) belongs to the family Zingiberaceae, is a perennial rhizomatous shrub native to Southern Asia [20,31], In India it is popularly known as “Haldi” and is extensively cultivated in all parts of India [7]. Its rhizomes are oblongonate, pyriform, often short branched and they are house hold remedy in Nepal [12]. As a powder called turmeric, it has been in continuous use for its flavoring, as a spice in both vegetarian and non-vegetarian food preparations and it also has digestive properties

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Table 1
Phytochemical analysis of various extracts of *Curcuma longa* (rhizome).

S. no.	Secondary metabolites	Name of test	Various extracts of *Curcuma longa*				
			Petroleum ether	Benzene	Chloroform	Methanol	Water
1	Alkaloid	Mayer's test	–	–	–	+	+
		Hager's test	–	–	–	–	–
2	Tannins and phenolic compounds	Ferric chloride test	+	+	+	+	+
		Vanillin hydrochloride test	+	+	+	+	+
3	Protein	Ninhydrin test	–	–	–	–	–
		Biuret test	–	–	–	–	–
4	Flavonoids	Shinoda test (magnesium hydrochloride reduction test)	+	+	+	+	+
		Alkaline reagent test	+	+	+	+	+
5	Steroids and triterpenoids	Salkowski test	–	–	–	–	–
		Libermann–Buchard test	–	–	–	–	–
6	Glycosides	Legal test	+	+	+	+	+
		Sodium nitroprusside test	+	+	+	+	+
7	Carbohydrates	Benedit's test	+	+	+	+	+
		Fehling's test	+	+	+	+	+

[15]. The objectives of this study was to evaluate the antimicrobial activity of the extracts from turmeric (*C. longa* L.) against common nosocominal pathogen *S. aureus.*

2. Material and methods

2.1. Plant material and extraction

The rhizome of *C. longa* (turmeric) was purchased from market and 25 gm of dry powder was packed in Soxhlet apparatus for extraction of respective soluble bioactive molecules from the rhizome by the use of different solvent (petroleum ether, benzene, chloroform, methanol and water). Fractions containing volatile solvents, were concentrated with the help of rotary evaporator (rota vapor) under reduce pressure. The concentrated extract was unloaded to sterilized collecting tube.

2.2. Test microorganisms

The standard strains of *S. aureus* ATCC 6571 used as test organisms were obtained from All India Institute of Medical Sciences, New Delhi. While clinical isolates were obtained from clinical material submitted for diagnostic Microbiology, Department of Microbiology, S.N. Medical College, Agra. Cultures of bacteria were grown on nutrient broth (Hi Media, Mumbai) at 37 °C for 12–14 h and were maintained and preserved on nutrient agar slants (Hi Media, Mumbai) at 4 °C prior to use.

2.3. Phytochemical analysis of the plant extract

Preliminary phytochemical screening of plant was done following the standard procedures adapted by the various workers [11,17,18]. The extracts were subjected to phytochemical tests for determination of plant secondary metabolites such as tannins, saponins, steroid, alkaloids and glycosides in accordance with [17].

2.4. Antibacterial activity

To check the presence of antimicrobial substance, the antimicrobial susceptibility tests were performed by standard disc diffusion method [8]. In this method, antibiogram patterns were studied for different extracts of *C. longa (*rhizome) viz petroleum ether extract, benzene extract, chloroform extract, methanol extract and aqueous extract for comparing three concentrations 1250 μg/disc (50 mg/ml), 2500 μg/disc (100 mg/ml) and 5000 μg/disc (200 mg/ml) based on available literature [22,9]. Empty sterile discs having a diameter of 6 mm were impregnated with 25 μl of each serial dilution of extract solution. These impregnated discs, now contain different concentration (1250, 2500, 5000 μg/disc) respectively of extract and then incubated for 15 min for proper diffusion of extract and placed onto nutrient agar surface spread with 0.1 ml of bacterial culture (standardized to 0.5 McFarland standards ($106\,cfu.ml^{-1}$). 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. Control discs contained DMSO only. For comparison, standard antibiotic gentamycin inhibiting bacterial cell wall biosynthesis was included in the assay. The antibacterial spectra showing zone of inhibition in millimeters and calculated as percentage by taking gentamycin as positive control with 100% inhibition.

Table 2
Antimicrobial susceptibility test of different fractions of *Curcuma longa* rhizome extract against *S. aureus* ATCC 6571 and clinical isolates.

Test pathogens	Zone of inhibition (mm)															
	Petroleum ether			Benzene			Chloroform			Methanol			Aqueous			Gentamycin control
	50 mg/ml	100 mg/ml	200 mg/ml	50 mg/ml	100 mg/ml	200 mg/ml	50 mg/ml	100 mg/ml	200 mg/ml	50 mg/ml	100 mg/ml	200 mg/ml	50 mg/ml	100 mg/ml	200 mg/ml	
S. aureus ATCC 6571	19	20	22	17	17	18	9	13	15	12	20	22	18	12	14	25
S. aureus (CI–I)	15	18	19	9	11	12	10	14	16	15	19	20	19	11	12	20
S. aureus (CI–II)	15	16	18	10	11	13	12	15	17	20	20	21	10	16	12	21