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Ethanol extract of *Curcuma longa* leaf, a potential drug candidate against *Bacillus* species mediated infections

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Abstract

Several studies have exposed the antimicrobial and antioxidant activities of *Curcuma longa*, widely used in food industry as a colorant, among other functions. This study was implemented to examine the antimicrobial activity of *Curcuma longa* leaves against six *Bacillus species*. Antimicrobial activities of three different extracts of *Curcuma longa* leaves on *Bacillus species* were tested on the basis of disk diffusion method and the zone of inhibition was measured. The ethanol extract of the plant leaf produced maximum zone of inhibition against *Bacillus cereus* Xb21 (12.50mm) followed by *Paenibacillus sp.* BF38 (11.10mm), *Bacillus simplex* Xb17 (10.40mm), *Bacillus megaterium* Hb42 (9.15mm), *Paenibacillus sp.* L32 (8.00mm) with the exception of *Terribacillus sp.* 3LF showed resistance, whereas the ethyl acetate and hexane extracts did not exhibit against all the tested bacteria. The chloroform extract of *Curcuma longa* leaves exhibited against only *Bacillus simplex* Xb17 (14.45mm) and *Paenibacillus sp.* BF38 (12.25mm).The MIC (minimum inhibitory concentration) values of ethanol extract against tested bacteria were almost 15.625 µg/10µl.The results suggest that the ethanol extract of *Curcuma longa* leaves could be considered as potentially effective antimicrobial agents against tested *Bacillus species*.

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Introduction

Bacillus species are a diverse group of bacteria widely spread in soil and the aquatic environment. This group of bacteria acclimatise easily to diverse territories caused by their ability to form spores and withstand a range of variable environmental conditions (Rahman *et al.,* 2013; Hasan *et al.,* 2013; *Priest et al.,* 1988).The antimicrobial agent is that kills microorganisms are called as microbicidal or inhibits their growth are known as microbiostatic. Use of substances with antimicrobial properties is known to have been common practice for over 2000 years.

Turmeric (*Curcuma longa*), a perennial herb and member of the Zingiberaceae family that is cultivated extensively in South Asia and other countries with a tropical climate. The extracts of turmeric roots has traditionally been used as an insect repellent, antimicrobial (Rudrappa and Bais, 2008), antidiabetic (Mohmed *et al.,* 2009), rheumatism, bodyache, skin diseases, intestinal worms, diarrhea, intermittent fever, hepatic disorders, biliousness, urinary discharges, dyspepsia, inflammations, constipation, leukoderma, amenorrhea and colic inflammatory disorders (Villages *et al.,* 2008).The most important compounds responsible for the antioxidant activity of turmeric are phenolic compounds, such as curcuminoid dyes and essential oils (Antunes *et al.,* 2012). Traditionally, the leaves of *C. longa* extensively used in culinary preparation are aromatic and contain essential oil. *C. longa* leaves oil bestowed with medicinal values has been used for treatment of various ailments and many of its therapeutic properties have been experimentally validated including its antimicrobial activity (Apisariyakul *et al.,* 1995; Tripathi *et al.,* 2002). Therefore, much attention has been focused on application of plant derived antimicrobials to control pathogens in foods. Consequently, alternative additives are needed, which possess antimicrobial activity and cause no health problems (Parvin *et al.,* 2013; Morshed *et al.,* 2011; Fayzunnessa *et al.,* 2011; Dorman and Deans, 2000).There are many literatures reporting the medicinal values of *Curcuma longa*, but there is little scientific evidence for further using this plant commercially. Therefore, an attempt was made to isolate curcuminoids from *Curcuma longa* leaves for their antimicrobial activities.

Materials and methods

Plant material and preparation of the plant extracts

Fresh leaves of *Curcuma longa* were collected from the local area of Kushtia district in Bangladesh. The sample was identified by Botanist. Fresh leaves were cleaned and cut into small pieces and air dried for 2 days. The dried sample was again dried in a hot air oven at 50°C for 24 hrs, then pulverized by a mechanical grinder and passed through a 20 mesh sieve. 10 g powdered was successively extracted with ethanol, ethyl acetate, chloroform, and hexane using a Soxhlet apparatus. The extraction was carried out for 24 hrs at room temperature with mild shaking. All the extracts were filtered and concentrated at 35°C under reduced pressure. The filtered extracts were concentrated in rotary vacuum evaporator at below 50°C and stored at 4°C for further experiment.

Bacillus species used

Six test strains viz. *Bacillus megaterium* Hb42, *Bacillus simplex* Xb17, *Terribacillus sp.* 3LF, *Bacillus cereus* Hb21, *Paenibacillus sp.* L32, and *Paenibacillus sp.* BF38 were employed for the present study. The microorganisms were maintained by sub-culturing and used at regular intervals in nutrient agar medium.

In Vitro Antibacterial activity assay

In vitro antibacterial activities of the test samples were carried out by disc diffusion method (Barry, 1976; Hasan *et al.,* 2013). The solutions of known concentration (µg/10µl) of test samples were prepared by dissolving exact amount of samples into measured volume of different solvent. The sample discs were made by soaking the sterilized filter paper discs (6mm diameter) with known volume of the test samples. Then the extract soaked discs were placed carefully over the nutrient agar media spreading with bacterial strains using the sterilized forceps. The prepared plates were incubated at 37°C overnight.

After the incubation, the zones of inhibition were visualized surrounding the discs. The antibacterial activities were assessed by measuring the zone of inhibition in millimeter (mm) against the test organisms. Standard Neomycin (30 µg/µl) was used as positive control for comparison of the antibacterial activity. The experiment was carried out in triplicate and read the mean value.

Determination of minimum inhibitory concentration (MIC)

It was described in details previous study on this test. Minimum inhibitory concentrations (MIC) of the ethanol, ethyl acetate, chloroform and hexane extracts were determined using serial dilutions (500 µg/10µl to 62.5 µg/10µl) of different extracts in ethanol, ethyl acetate, chloroform and hexane solvent against six tested microorganisms by agar well diffusion method (Hasan *et al.,* 2013; Reiner, 1982). Freshly grown bacterial strains 100 µl (10^6cells/ml) in nutrient broth was inoculated in tubes with nutrient broth supplemented with different concentrations (10.0 to 1000.00 µl) of the stock extract (1 mg/ml) and antibiotic respectively and incubated for 24 hrs at 37°C. Presence of turbidity denoted presence of microorganism in the test tube after the period of incubation, whereas the complete absence of any turbidity indicates complete inhibition of microbial growth. The test tube with the lowest dilution with no detectable growth by visual inspection was considered the MIC.

Data analysis

All data were measured average value of three replicates and standard error (±).Results were subjected to ANOVA using Statistical Packages for Social Sciences (SPSS version 20.0) and the means separated using least significant differences (LSD) at 5% level of significance

Results

The results of antimicrobial activity of ethanol, ethyl acetate, chloroform and hexane extracts of *Curcuma longa* leaves against different *Bacillus species* are presented on the Table 1.

Table 1. Determination of antimicrobial activity of ethanol, ethyl acetate, chloroform and hexane extracts of *Curcuma longa* leaf against different bacterial strains.

Bacterial strain	Zone of inhibition (mm)				
	Leaf extracts of *Curcuma longa* (500µg/10µl)				Standard (30µg/µl)
	Ethanol	EA	Chloroform	Hexane	Neomycin
Bacillus megaterium Hb42	9.15±0.23*	-	-	-	10.60±0.32
Bacillus simplex Xb17	10.40±0.14*	-	14.45±0.08	-	12.23±0.29
Terribacillus sp. 3LF	-	-	-	-	-
Bacillus cereus Xb21	12.50±0.30*	-	-	-	12.00±0.00
Paenibacillus sp. L32	8.00±0.00*	-	-	-	9.20±0.38
Paenibacillus sp. BF38	11.10±0.46*	-	12.25±0.17	-	11.00±0.00

Data were measured in mm and represented as mean ± SD of triplicate. EA: Ethyl acetate. (*) indicates significance value P<0.005.

Table 2. Determination of minimum inhibitory concentration (MIC) of different leaf extracts of *Curcuma longa*.

Bacterial strain	Minimum inhibitory concentration(MIC)			
	Organic extracts of *Curcuma longa* leaf			
	Ethanol	EA	Chloroform	Hexane
Bacillus megaterium Hb42	62.5	-	-	-
Bacillus simplex Xb17	62.5	-	62.5	-
Terribacillus sp. 3LF	-	-	-	-
Bacillus cereus Xb21	62.5	-	-	-
Paenibacillus sp. L32	62.5	-	-	-
Paenibacillus sp. BF38	125	-	125	-

MIC of different organic extracts (values in µg /10µl).

The zone of inhibition was measured in millimetre. In accordance with concentration of 500 µg/µl, the highest zone of inhibition for ethanol extract was *Bacillus cereus* Xb21 (12.50±0.30 mm),followed by *Paenibacillus sp*. BF38 (Figure 2) (11.10±0.46 mm), *Bacillus simplex* (Xb17) (10.40±0.14 mm) (Figure 2), *Bacillus megaterium* (Hb42) (9.15±0.23 mm), *Paenibacillus sp*. L32 (8.00±0.00 mm),whereas no zone of inhibition was shown by *Terribacillus sp*. 3LF.It was found for chloroform extract, the zone of inhibition for *Bacillus simplex* Xb17 and *Paenibacillus sp*. BF38 were 14.45±0.08 mm and 12.25±0.17 mm respectively, however, no zone of inhibition was shown rest of others *Bacillus species*. It has been seen that Ethyl acetate and hexane extract did not produce the activity against all of the tested bacteria (Figure 1).

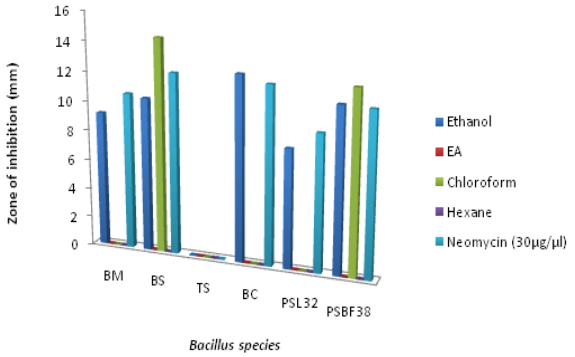

Fig. 1. The comparison of antimicrobial activity of *Curcuma longa* leaf extracts and standard antibiotic (Neomycin 30µg/µl) against *Bacillus species*. EA: Ethyl acetate, BM: *Bacillus megaterium* Hb42, BS: *Bacillus simplex* Xb17, TS: *Terribacillus sp*. 3LF, BC: *Bacillus cereus* Hb21, PSL32: *Paenibacillus* sp. L32, PSBF38: *Paenibacillus* sp. BF38.

The minimum inhibitory concentration (MIC) test was done by broth dilution method to discern the lowest concentration of extract required for the complete inhibition of bacterial growth. The ethanol and chloroform extract of *Curcuma longa* leaves exhibited MIC of 125 µg /10µl for *Paenibacillus sp*. BF38, whereas MIC of 62.5 µg /10µl for *Bacillus megaterium* Hb42, *Bacillus simplex* Xb17, *Bacillus cereus* Xb21, *Paenibacillus sp*. L32 was exhibited by ethanol extract of the plant and chloroform extract for *Bacillus simplex* Xb17 (Table 2).

Discussion

In the present study, ethanol extract of *Curcuma longa* leaves showed significant antimicrobial activity against most of the tested organisms ($p<0.005$) except *Terribacillus sp*. 3LF.This is because of the fact that some essential oils contain active components that influence certain metabolic functions of microbial cells. Wilkins and Board suggested that antimicrobial activity of oils may be due to impairment of variety of enzymes systems that are involved in the production of energy or synthesis of structural components in the microbial cells (Wilkins and Board, 1989). The differences in the sensitivity of food borne pathogens may be due to differences in methods used in study (Parvin *et al.,* 2013; *Kumar et al.,*1997).

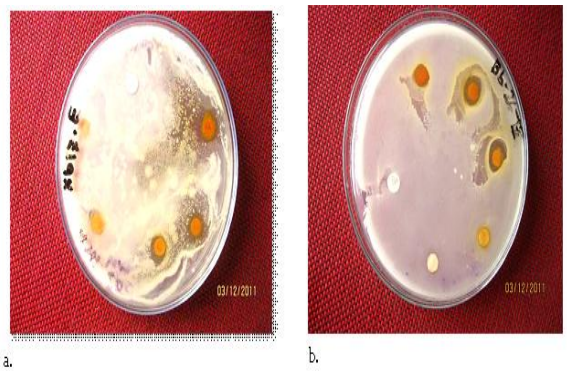

Fig. 2. Ethanol extract of Curcuma longa leaf produce zone of inhibition at different concentrations against *Bacillus species* (a. *Bacillus simplex* Xb17, and b. *Paenibacillus sp*. BF38.).

The plant extracts were described with standard antibiotic i.e. neomycin (30 µg/µl) and negative control (only solvent absorbing disc).The negative control exhibited no activity against all tested bacteria. The standard antibiotic showed significant antimicrobial activity against most tested bacteria but did not produce antimicrobial activity against only *Terribacillus sp*. 3LF.

Among the tested extracts, the most active was ethanol extract, and chloroform, while no inhibitory action of ethyl acetate and hexane extract was observed. In comparison with the present study, it is supported that crude ethanol extracts of

Curcuminoids of *Curcuma longa* from the varieties of Kasur, Faziabad and Bannu areas the antibacterial activity against three *Bacillus species* and one Azatobacter resulted effective activity with Kasur variety than others and *B. subtilis* was the most sensitive to Curcuminoids and oils of turmeric (Naz *et al.,* 2010).

Similar work on antibacterial studies using root extract of *C. igneus* on *P. aeruginosa, Klebsiella pneumonia, Proteus vulgaris* and *Salmonella* sp. was reported (Nagaranjan *et al.,* 2011). Antimicrobial activities of different medicinal plants have been described worldwide by many researchers (Morshed *et al.,* 2011; Samy, 2005; Palombo and Semple, 2001; Antunes *et al.,* 2012; Rahman *et al.,* 2013). Antibacterial activity of *Curcuma longa* varieties against different strains of bacteria (Kumar *et al*., 1997; Luthra *et al*., 2001), rhizome extract of Acorus (Sabitha *et al*., 2003), leaf extract of *Mikania triangularis(Cruz et al.,* 1996), root and leaf extract of *Withania somnifera* (Mahesh and Satish, 2008) have been reported.

Conclusions

In conclusion, the findings of the present study clearly indicate that the ethanol extract of *Curcuma longa* leaves possess the antimicrobial activity like that of the rhizome which has proved evidence for its antimicrobial potential. The ethanol might be a good solvent for extraction *of Curcuma longa* leaves. Further studies may recommend for the isolation of bioactive constituents and biological assay methods for the standard drug preparations.

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