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INVESTIGATION OF ANTIMICROBIAL AND ANTI-INFLAMMATORY ACTIVITY OF *CURCUMA LONGA*

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ABSTRACT: Turmeric (*Curcuma longa*) is a rhizomatous herbaceous perennial plant used as a food additive. It has been reported that rhizome of this plant have antibacterial, antifungal, anti-inflammatory, antioxidant and antitumor property. Methanol extract of Rhizome of *Curcuma longa* was investigated here to see the antimicrobial actions and anti-inflammatory effect. During the extraction process a purified single compound (D1) was isolated and investigated for its antimicrobial activity. Significant antimicrobial activity than penicillin were found for 500µg *C. longa* extract. Anti-inflammatory action of *C. longa* was also assessed using mice models. The purified compound D1 fraction showed antimicrobial action in 50µg concentration. Our study reveal that *C. longa* has antimicrobial activity against various gram positive and gram negative bacteria where curcumin may not be the only compound that is responsible for the antimicrobial activity. On the other hand, *C. longa* extract had shown significant anti-inflammatory action.

INTRODUCTION: Plants used as traditional medicines are a source of wide range of substances which can be useful to treat infectious or other serious diseases and are used for medicinal purposes from long since [1]. Huge research works are conducting now to screen potential antimicrobial or other therapeutically useful substances from plants. *Curcuma longa* one of the widely used traditional medicine source (Family-Zingiberaceae, Popular in Bangladesh as Holud) is a perennial herb with pupy, orange, tuberous roots cultivated extensively in Asian countries like Bangladesh, India and China [2, 3]. Zingiberaceae family constitutes a vital group of rhizomatous medicinal and aromatic plants characterized by the presence of volatile oils and oleoresins. Generally, the rhizomes and fruits are aromatic, tonic and stimulant; occasionally they are nutritive. Some are used as food as they contain starch in large quantities while others yield an astringent and diaphoretic juice.

The important genera coming under Zingiberaceae are *Curcuma, Kaempferia, Hedychium, Amomum, Zingiber, Alpinia, Elettaria* and *Costus* [4].

Cucuma longa (turmeric) is used extensively as spice, preservative and coloring agent in India, China & South East Asia [5]. It is also used locally for home made remedy for various diseases. Curcumin (diferuloylmethane), the main yellow bioactive component of turmeric has been shown to have a wide spectrum of biological actions: Antioxidant effects, Hepatoprotective effects, Anti-inflammatory effects, Anticarcinogenic effects, Antimicrobial effects and Cardiovascular effects [2, 6-8]. Here, we have re-investigated the Antimicrobial activity and Anti-inflammatory activity of crude *Curcuma longa* and searched for new compounds other than the known compounds using chromatography.

Anti-diarrhoeal activity of *Curcuma longa* has also been studied using animal models.

METHOD AND MATERIALS:

Plant Material. *Curcuma longa* (rhizome) was collected from a village in the district of Comilla and was identified by Bangladesh National Herbarium (BNH) Reference no:-35157, and identification no.-BK-01. The rhizomes were washed by fresh water. All dust and other contaminants were removed and the rhizomes were cut into very thin slices. The slices were then taken in a fresh big sheet of white paper and sun dried for several days until the slices become fully dried and crispy. The dried slices of turmeric were then powdered using the grinder.

Extraction and isolation. The sample of *Curcuma longa* was extracted by cold extraction process [9]. About 169 gram of powdered turmeric was taken in a beaker, then dissolved with methanol (99.99%). Total 500 ml of methanol was added in three different times. And extraction of 213.4 gm of methanol + turmeric extract was collected by filtering the mixer of turmeric and methanol. Evaporation of the methanol from turmeric extract was done by rotary evaporator. (RPM: 70 and Temperature: 37-45^{o}C).

Chromatographic Analysis. Two different types of chromatography were performed in this study.

(a) Thin Layer Chromatography (TLC) and;
(b) Column Chromatography (CC).

The thin layer chromatography (TLC) was done using normal phase TLC, where TLC plate with SiO_2 gel was used in stationary phase [10]. During column chromatography (CC), a gravity column was made by using column grade silica gel (SiO_2) and total 100 gm of silica gel (SiO_2) was used [11].

At first the column was rinsed by n-Hexane, and then purified cotton was placed in the bottom of the column tube, and then the silica gel was added to the n-hexane, and poured in the tube. Sample with some silica gel and some chloroform was mixed and after then the chloroform was evaporated by rotary evaporator, and then poured on the silica column settled in the column tube. With the help of different solvent system (supplementary **table 1 & 2**) we have separated different compound based on polarity difference of those compound in the sample. The compound in the sample showed different bands of color based on polarity with different solvent system, the separated band was collected in 57 different test tubes, and TLC of each fraction was done for confirming the number of compounds in a band separated. Four different spots were found with same R_f value in nine test tubes when TLC was done. The contents of test tubes were mixed and a second column chromatography was performed, where four different fractions named D1, D2, D3 and D4 were collected. Among these fractions only D1 fraction showed significant antimicrobial activity, therefore included in further analysis.

Spectroscopic Analysis: For FTIR (Fourier Transform infra-red) analysis 1.5 mg (0.0015 g) D-1 was mixed with 200 mg (0.20 g) of dried, Spectrophotometric-grade KBr and sample dick was prepared [12]. Finally UV spectra was analyzed at a range between (200-800) nm using;

(a) 1µg of sample (D1) dissolved in Methanol (HPLC grade) only,

(b) 1µg of sample dissolved in Methanol and NaOH, and;

(c) 1µg of sample dissolved in Methanol and HCl.

Bioassays:

Antimicrobial Test: Crude extract of turmeric was measured in analytical balance, and dissolved in chloroform in a specific concentration 10 µg/µL. The liquid form of sample was made for optimal concentration of dose in each paper disk. The desired disk concentration was 500 µg, 200 µg, 100 µg. 14 different bacterial strains were used in our study to perform antimicrobial test (**Table 1**). All the strains were cultured from the mother strains as a stock culture in vials, and then they were re-cultured in petri dishes, then the paper disks were prepared with specified concentration of sample. Prepared disks were put on the 3 specified places in the plate with positive control (Penicillin paper disk). The samples were given at 500 µg, 200 µg, and 100 µg concentrations for crude and for single compound (D1) fraction 50 µg, 20 µg and 10 µg of concentrations were used.

TABLE 1: BACTERIAL SPECIES USED IN ANTIMICROBIAL ASSAY

Bacteria	Class
Bacillus subtilis	Bacilli
Escherichia coli	Gamma proteobacteria
Salmonella typhi	Gamma proteobacteria
Candida albicans	Saccharomycetes
Pseudomonas aureus	Gamma proteobacteria
Saccharomyces cerevisiae	Saccharomycetes
Streptococcus aureus	Coccus
Vibrio parahaemolyticus	Gamma proteobacteria
Shigella boydii	Gamma proteobacteria
Shigella dysenteriae	Gamma proteobacteria
Sarcina lutea	Clostridia
Salmonella paratyphi	Gamma proteobacteria
Bacillus cereus	Bacilli
Vibrio mimicus	Gamma proteobacteria

Anti-inflammatory Test: The methanol extract of *C. longa* was used for anti-inflammatory test. The mice were 4-5 weeks old (weight: 23-28 gram) was divided into 5 groups for experimental use. They were purchased from the animal research branch of International Centre for Diarrheal Diseases Research, Bangladesh (ICDDR, B) [13]. The mice were kept in standard condition and were given ICDDR, B formulated rodent food. The five groups were marked as (+)ve control, (–)ve control, sample-1(500 mg/kg), sample-2 (250 mg/kg), sample-3 (125 mg/kg).

At zero hour: test samples, Control (1% Tween-80 solution in saline) and Aspirin were administrated orally by means of a long needle with a ball shaped end. After 40 minutes acetic acid (0.7%) was administrated (0.1 ml/10 gm of body weight) to each of the animal of all groups. The forty minutes interval between the oral administration of test materials and intra- peritoneal administration of acetic acid was given to assure proper absorption of the administered samples. Five minutes after the administration of acetic acid, number of writhing were counted for each mouse for fifteen minutes.

RESULT: Antimicrobial test when performed with different concentration of *C. longa* extract the highest zone of inhibition found for *Shigella boydii* (16 cm) at 500 µg of dose and lowest zone of inhibition was nil(0 cm) for *Salmonella typhi* and *Saccharomyces cerevisiae* culture compared to penicillin 10 IU disk. The overall antimicrobial activity was quite indicative as other 12 bacterial species were significantly inhibited by *C. longa* 500 µg dose (**Figure 1**). Another antimicrobial test with D1 compound also showed representative inhibition of 13 different bacterial species in 50 µg concentration (**Figure 3**).

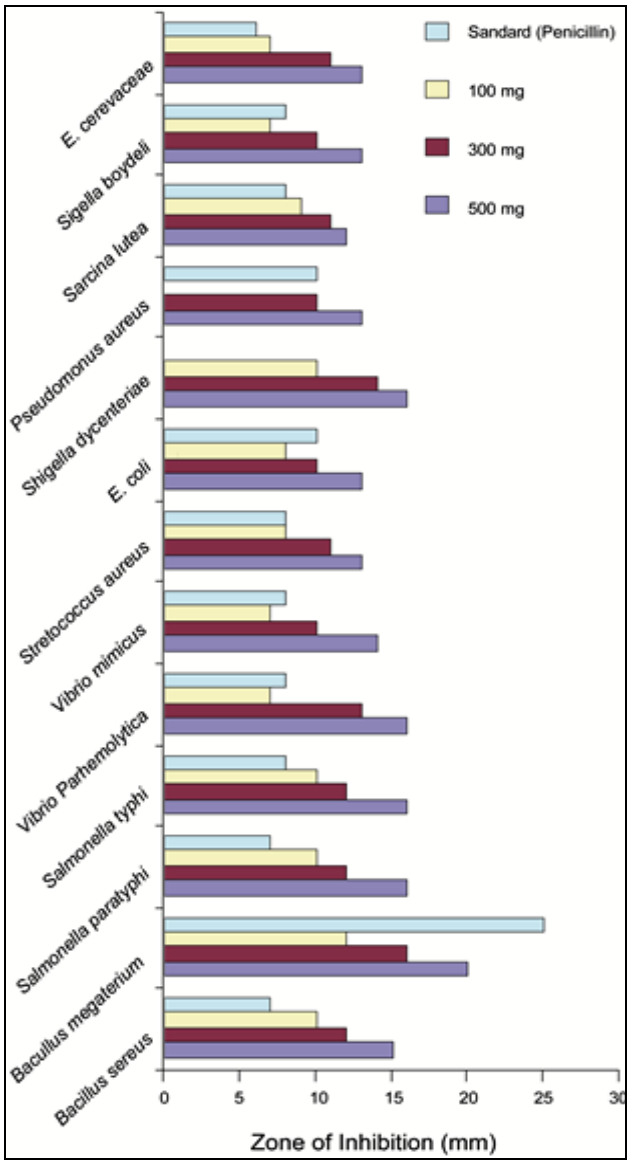

FIGURE 1: ANTIMICROBIAL ACTIVITY OF *CURCUMA LONGA* EXTRACT. Penicillin was used to compare the zone of inhibition of *Curcuma longa* extract. in many cases extract has shown better performance (larger zone of inhibition) than penicillin itself. color for different strength is shown in the figure at top-right corner**.**

The crude methanol extract showed significant result in the anti-inflammatory effect test. The significance value was found 0.0001, which is very significant at a dose of 500mg/kg of body weight, and in 250mg/kg body weight the P value is 0.0003 (**Figure 2**). As *C. longa* shown very significant anti-inflammatory activity, so further study at molecular level needed to find out the adjunct molecular mechanism and by doing this many novel activity could be found.

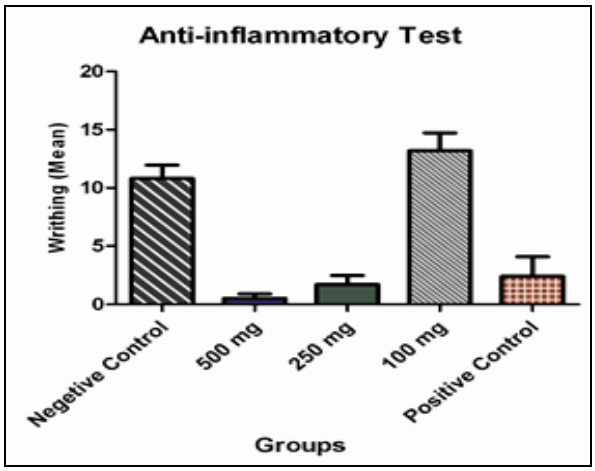

FIGURE 2: ANTI-INFLAMMATORY TEST OF *CURCUMA LONGA* EXTRACT. Five different groups of mice models were used for anti-inflammatory test of Curcuma longa. Acetic acid was used to induce inflammation, Normal saline was used as negative control and Aspirin was used as positive control here.

In the IR spectra peak for –OH was seen at 3466 cm^{-1}, presence of olefinic proton was indicated by the peak at 2941 cm^{-1}, no strong peak for carbonyl group was seen. Peak at 1602 cm^{-1} and 1490 cm^{-1} indicated the aromatic ring in the compound. UV spectra of the compound D1 showed strong absorption at 420 nm, (absorbance 0.091), in (D1 + methanol) preparation, which confirm conjugated system in the compound. Then another sample was prepared with (D1 + Methanol + NaOH), Peak was seen at 478 nm (absorbance 0.082), has shown hypsochromic shift, which indicate aromatic –OH group is present and the shifting is due to ionization of aromatic –OH group. In another sample (D1 + Methanol + HCl) peak was seen at 426nm (absorbance 0.088) but no significant shifting of was seen.

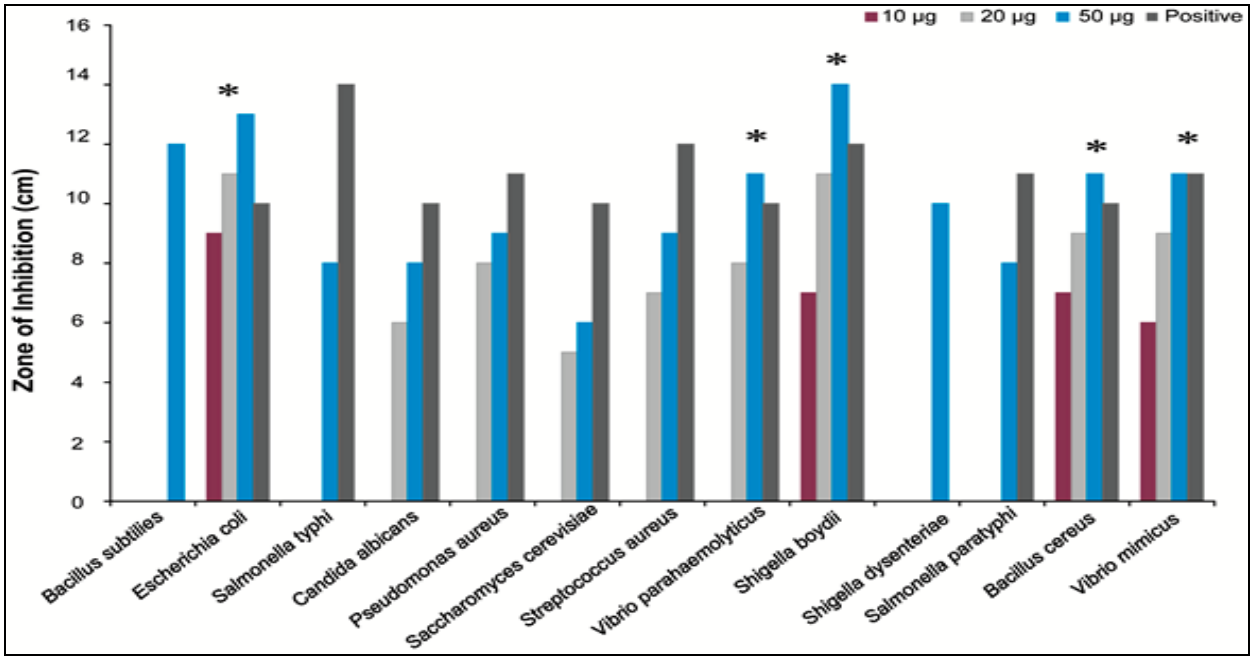

FIGURE 3: ANTIMICROBIAL ACTIVITY OF D1 FRACTION. ANTIMICROBIAL ACTIVITY OF D-1 FRACTION IS SHOWED WITH DIFFERENT STRENGTH. Significant zone of inhibition found with 50µg D1 fraction is marked with star. Penicillin was used to compare the zone of inhibition as positive sample.

DISCUSSION: Crude *C. longa* extract has shown significant anti-inflammatory and antimicrobial action. Among the selected strains of bacteria used in this study only *Bacillus megaterium* didn't show any significant inhibition when treated with *C. longa* extract. Other thirteen bacterial species showed larger zone of inhibition than standard (Penicillin), when 500 µg *Curcuma longa* was used. Curcumin is a compound found in *Curcuma longa* has been reported earlier for its wide spectrum antimicrobial activity by Rudrappa and Bais in 2008 [14]. Five bacterial *species Shigella boydeii, shigella dycenteriae, Escherichia Coli, Salmonella typhi* and *salmonella paratyphi* in this study used is responsible for intestinal infection [15]. Therefore, an anti-diarrheal test was conducted on a mice model (Swiss Albino), but didn't found any significant result so the result of that test is not included here. Furthermore, when zone of inhibition of D1 fraction compared with penicillin at 3 different concentration significant reduction of bacterial growth was found against five bacterial species; *Escherichia coli, Vibrio parahaemolytica, Shigella boydii, Bacillus cereus* and *Vibrio mimicus* (Figure 3).

No significant inhibitory zone was found in *Salmonella typhi* and *Salmonella paratyphi* bacterial culture. This result is different than the previous antimicrobial study, thus we concluded that, there may be a new compound in that fraction other than curcumin [16-17].

Anti-inflammatory study of *C. longa* were performed with 3 different strengths where 500 mg *C. longa* extract showed significant anti-inflammatory effect with P value 0.0001 (Figure 2). Anti-inflammatory and immunomodulatory action of *C. longa* is thought to be due to curcumin and turmerones [14-18].

Infra red and UV visible spectroscopy were done for D1 fraction. From IR spectroscopic analysis of D1 compound –OH peak was found at 3466 cm^{-1}, presence of Olefinic proton was indicated by the peak at 2941 cm^{-1}. Peak at 1602 cm^{-1} and 1490 cm^{-1} indicate the presence of aromatic ring in the compound and no strong peak for carbonyl group was seen. Chemical analysis of D1 fraction through UV also confirmed the presence of –OH group, conjugated bond and aromatic ring in that compound.

CONCLUSION: *C. longa* had shown wide spectrum of antimicrobial activity and significant anti-inflammatory effect in our study. Isolation and chemical investigation of D1 fraction with its anti-microbial assay indicate that, this could be a new compound other than the compounds already found in *C. longa*. Further chemical and biological study needed to establish the actual structure and function of the compound, which was beyond the scope of our laboratory facilities. However, antidiarrhoeal activity of C. longa is not found to be significant.

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