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Antimicrobial Potency of the Leaf – Stalk Extract Of Curcuma longa (LINN)

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ABSTRACT: *The methanolic extract of the leaf-stalk of curcuma longa LINN, was tested for its minimum Inhibitor concentration (MIC) against Gram positive-staphylococcus aureus, Bacillus pumilus, Bacillus subtilis, klebsiella pnemoniae, bacillus cereus, streptococcus pneumoniae, Lactobacillus arabinosus and gram negative E.coli, shigella dysenteriae, shigella sonnei, shigella boydii, salmonella typhimurium, proteus mirabilis, and Vibrio cholerae strains, further, the ones of inhibition produced by the crude extract against four selected bacterial strains were measured and compared with those produced by the standard antibiotic ciprofloxacin against the same bacterial strains.*

INTRODUCTION:

The different parts of curcuma longa Linn Plant (Family-Zingiberaceae) are known for their various medicinal properties (1-13) The present investigations were undertaken to test the anti-microbial activity and to find out the minimum Inhibitory concentration (MIC) of the leaf stalk extract of this plant against some gram positive and gram negative bacteria. Further the anti-microbial potency of the extract was compared with that of the standard antibiotic ciprofloxacin against a selected number of bacterial strains.

MATERIALS AND METHODS:

Fresh plants were collected, the leaf stalks were separated from the plants and identified b the expert of Pharmacognosy of our department the leaf stalks were washed, cut into small pieces each of 2.5 to 3.0 cm in length, sundried and ground to a coarse powder in a grinder.

METHOD OF EXTRACT PREPARATION:

The coarse powder of the leaf-stalks (115 gm) was extracted in a soxhlet apparatus with methanol and the solvent was removed b evaporating on a heating mantle by taking care tat the temperature did not rise above 40^{o}C. A semi solid, dark viscous crude extracts of the part was obtained. This crude extract was tested for its anti-microbial activity against various; bacterial strains, like *S. aureus B. Pumilus, B subtilis, B.cereus, streptococcus pneumoniae, E. Coli, shigella dysenteriae, shigella sonnei, shigella boydii, salmonella typhimurium, proteus mirabilis, and Vibrio cholerae and Lactobacillus arabinosus*. These bacterial strains were collected from the Department of Pharmaceutical Technology, Jadavpur University, central Drugs Laboratory, Calcutta, S.C.B. Medical College, cuttack

Orissa, and Institute of Microbial Technology (IMT), Chandigrah. All the strains used were pure cultures, preserved as stab slant cultures at a temperature of 4^{o}C. The strains were clinical isolates obtained form different parts of the country.

DETERMINATION OF MINIMUM INHIBITORY CONCENTRATION (MIC) BY SERIAL DILUTION TECNIQUE:

A stock solution (25ml) of the crude extract of 1 mg/ml concentration was prepared b dissolving 25 mg of the same in ethylene glycol ethylene glycol did not show any activity as such (14).

Calculated volumes of this stock solution were dispensed in a series of McCartney bottles previously containing calculated volumes of sterile cooled molten nutrient agar media (40-45o) to prepare the final volume of 20 ml each, with dilutions of 3,5,10,25,50,100,150 and 200 µg/ml. These molten nutrient agar media containing various concentrations of the extract were poured and solidified on the sterile petridishes to give sterile nutrient agar plates, they were kept in the refrigerator for 24 ours for uniform diffusion of the extract trough the nutrient agar media. These plates were ten dried at 39^{o}C in the incubator for 2 hours prior to spot inoculation. One loopful (loop diameter:3mm) of an overnight grown peptone-water culture of each test organism served as a inoculum for the serial dilution technique. The back of each test plate was marked b checker board technieque for the location of ac inoculum and the test organisms were spot inoculated accordingly. The spot inoculated plates were incubated for 24 hours at 37^{o}C and the MIC values were determined.

DETERMINATION OF ZONES OF INHIBITION BY DISC DIFFUSION TECHNIQUE:

In this method, Ciprofloxacin, pure, was taken as the standard antibiotic for the comparison of the results. Stock solutions (each of 1 mg/ml concentration) of both the crude extract and the standard antibiotic were prepared. From these stock solutions 2 sets of four dilutions (50,100,150 and 200 µg/ml) each of curcuma longa leaf stalk extract (solvent: ethylene glycol) and ciprofloxacin I (solvent: distilled water) were prepared in sterile McCartney bottles. Anti-microbial potency was determined by Disc diffusion Assay method employing 24 hours peptone-water cultures of four test organisms. Sterile nutrient agar plates were prepared and incubated at 37^{o}C for 24 ours to check of contamination, if any. Each sterile nutrient agar plate was then flooded with the corresponding peptone-water culture of the test organism, dried for 30 minutes at 37^{0}C for after drying of the flooded plate, four filter paper discs (Whatman no 1) of 6 mm diameter were soaked in the four different dilutions of he crude extract and placed at the specific locations on the surface of the flooded plate, marked as quadrants at the back of the plate. The same technique was repeated in the case of the remaining test organisms for both the extract and the standard antibiotic. All the flooded plates with the corresponding filer paper discs on them were incubated at 37^{o}C for 24 hours and the diameters f the ones of inhibition were measured in mm and compared accordingly.

RESULTS AND DISCUSSIONS:

Results of the determination of MIC values of the *C. longa* Linn. Leafstalk extract have been recorded in Table-1 . It is evident form the results that the crude extract is active

against both gram positive and gram negative bacteria, but it is more active against gram negative bacterial strains at lower concentrations. Results of determination and comparison of zones of inhibition of the crude extract and the standard antibiotic, Ciprofloxacin, against four selected strains have been recorded in Table 2. The antimicrobial effect of the crude extract was found to decrease in the following order against different test organisms, *Shigella dysenteriae*, *E.Coli*, I, *Staphyclococcus aureus*, and *Bacillus cereus*, as evident form Table 2. The antimicrobial activity of the extract may be due to some antimicrobial substances present in the leaf-stalk of C. longa Linn. Further studies are going on in our laboratory to isolate the active principle responsible for the above effect.

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Table -1

Determination of MIC of the leaf extract of C. longa Linn. Against various bacterial strains:

Name of bacteria	Growth in nutrient agar containing different concentration of the extract (µg/ml)								
	0+	3	5	10	25	50	100	150	200
Staphylococcus aureus ML 267	+	+	+	+	+	+	+	±	-
S. aureus AM 8/98	+	+	+	+	+	IC	IC	-	-
S. aureus NCTC 7447	+	+	+	+	+	+	+	-	-
S. aureus 8531	+	+	+	+	+	+	+	-	-
S. aureus ATCC 2937	+	+	+	+	+	+	+	-	-
Bacillus pumilus 8241	+	+	+	+	+	+	+	-	-
B. subtilis CD/99/1	+	+	+	+	+	+	+	-	-
B.cereus var. mycoides	+	+	+	+	+	+	±	±	-
E.coil AM 8/98	+	+	IC	-	-	-	-	-	-
E.coil VC sonawave 3:37C	+	+	+	+	+	+	+	-	-
E.coil CD/99/1	+	+	+	+	+	+	±	±	-
Shigella dysenteriae 1	+	+	+	+	+	+	+	-	-
Shigella dysenteriae 2	+	+	+	+	+	+	+	-	-
Shigella dysenteriae 6	+	+	+	+	+	IC	±	-	-
Proteus mirabilis AM 8/98	+	+	+	+	+	+	+	-	-
Kelbsiella pneumoniae RM 8/98	+	+	+	+	+	+	+	±	-
Streptococcus pnemoniae NCTC 7465	+	+	+	+	+	+	+	-	-
Vibrio cholerae 865	+	+	+	+	+	+	IC	-	-
Lactobacillus arabinosus CD /99/1	+	+	+	+	+	+	+	±	-

Table -2

Determination of the diameters of ones of inhibition produced by the plant extract and their comparison with those of the standard antibiotic, ciprofloxacin, against the same selected bacterial strains:

Name of Bacteria	Extract (µg/ml)				Ciprofloxacin (µg/ml)			
	50	100	150	200	50	100	150	200
Shigella dysenteriae 1	7.5	8.0	9.5	11.0	18.0	19.5	20.0	23.0
Escherichia coli AM 8/98	7.0	8.5	9.5	11.0	.5	8.0	10.5	11.5
Staphylococcus aureus AM 8/98	6.5	7.0	80	9.5	9.5	10.0	12.0	13.5
Bacillus cereus var. mycoides	6.0	6.5	7.5	9.0	8.5	10.0	10.5	13.0

(all diameters were measured in millimeters)

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