The effect of *Curcurma longa* (Turmeric) rhizomes extracts on pathogenic bacteria In comparison with standard antibiotics

تأثير مستخلص السيقان الجذرية للكركم Curcuma longa (Turmeric) على البكتريا المرضية بالمقارنة بمضادات الحياة الاساسية

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Abstract:

Four extracts of *Curcuma longa* rhizomes (commonly known as turmeric widely used as spice and coloring agent and known for its medical properties) were evaluated for their anti- bacterial action against pathogenic bacteria of gram-negative (*Escherichia coli, Salmonella typhimurium*) and gram- positive (*Staphylococcus aureus, Bacillus cereus*) comparing with antibiotics (gentamycin, ampicillin and erythromycin). Essential oil which was extracted from turmeric found to be most active against pathogenic bacteria in comparison with other extracts (water, chloroform and methanol extract). Using 40 microgram/disc of essential oil of turmeric as a minimum inhibitory concentration posses significant activity on pathogenic gram-negative and gram- positive bacteria.

المستخلص :

اخذت اربع مستخلصات للسيقان الجذرية للكركم *Curcuma longa* الشائع تسميته بالـ (Turmeric) والذي يستعمل في التوابل وكمادة صبغية وله خصائص دوائية . قدرت فعاليته المضادة للبكتريا المرضية سالبة لصبغة كرام ، بكتريا القولون البرازية *Escherichia coli* والبكتريا السمية *Salmonella typhimurium* وعلى البكتريا المرضية موجبة لصبغة كرام ، *Staphylococcus aureus* والبكتريا السبورية *Bacillus cereus* مقارنة بمضادات الحياة الاساسية (الجنتامايسين Gentamycin الامبسيلين Ampicillin والارثرومايسين Erthromycin) الزيت الاساس الذي استخلص من الكركم وجد بانه الاكثر تأثير ضد البكتريا اعلاه اذا ما قورن بالمستخلصات الاخرى(المستخلص المائي ، مستخلص الكلوروفورم ومستخلص الميثانول) . 40 مايكرو غرام / قرص من الزيت الاساس للكركم كأدنى تركيز مثبط اظهر تأثيرا واضحا ضد البكتريا الممرضة سالبة لصبغة كرام والبكتريا الممرضة موجبة لصبغة كرام .

Introduction:

Curcuma longa which belongs to Zingiberaceae family, commonely known as turmeric is widely used as a spice and coloring agent in food and known for its medicinal properties [1, 2, 3,4, 5].

Turmeric is a perennial herb, cultivated extensively in Asia, India, China and other countries with a tropical climate. The rhizome is the portion of the plant used medicinally, it is usually boiled cleaned and dried yielding a yellow powder, dried *curcuma longa* is the source of the spice turmeric [6, 7, 8, 9].

Curcumin, the yellow color pigment of turmeric ,is produced industrially from turmeric oleoresin[10].Various curcuminoids have been isolated from the rhizome of cucurma longa, attributing a wide array of biological activities such as antioxidant, anti imlammatory, wound healing activity [6,11,8,9].

Natural products from some plants, fungi, bacteria and other organisms continue to be used in pharmaceutical preparations either as pure compounds or extracts [8].

The organolipitic properties of *C. longa* are odour, aromatic test warmly aromatic and bitter [9]. The active constituents of turmeric are the flavenoid, curcumin and volatile oils including turmerone,altantone and zingiberone. Other constituents include sugars, proteins and resins. The best active constituent is the dye curcumin, non toxic food which comprises 0.3 to 5.4% of raw turmeric [5,6]. The essential oil of *C.longa* inhibit the growth of variety of bacteria, parasites and pathogenic fungi, but neither was inactive against yeasts isolates. Curcumin has also been found to have moderate activity against pathogenic bacteria [1]. Ether and chloroform extracts of stem of *C.longa* were found to be fungestatic [6,12,13] Total extracts of the dried powdered combination of fruits of *M.charantia* (cucurbitaceae), *E.officinalis* (euphorbiaceae) and rhizomes of C .longa (zingiberaceae) showed better antibacterial activity compared to the individual plant ingredients alone[14]. The objective of this study was to determine the effect of various extracts of turmeric on pathogenic strains of gram- negative bacteria *Salmonella typhimurium* and *Escherichia coli* and gram-positive bacteria *Staphylococcus aureus* and *Bacillus cereus* by zone of inhibition assay and their effect was compared to various standard antibiotics (gentamycin, ampicillin and erythromycin) and to study the minimum inhibitory concentration of the most effected extract of rhizomes of curcuma longa on pathogenic bacteria.

Materials and methods:

Dry rhizomes of *C.longa* (turmeric) were purchased locally from local shops. The rhizomes (50gm) were ground finely and pestle by adding (200ml) water and subjected to steam distillation. The oily extract (fraction-A, 1.137gram) was collected and the residue in water was filtered. The filtrate was evaporated under vacuum to give water extract (fraction-D, 1.945gram). The residue was air-dried and left overnight in chloroform (100ml), filtered and re-extracted twice with chloroform (2 X 50ml). All chloroform extracts were combined and solvent was evaporated to give chloroform extract (fraction-B, 0.875gram). The residue left after chloroform was extracted with methanol to give the methanol extract (fraction-C, 2.6gram) , [2].

The gram-negative (*Salmonella typhimurium, Escherichia coli)* and the gram-positive bacteria (*Staphylococcus aureus ,Bacillus cereus*) were used as test organisms, they were obtained from College of Agricultural-food science and biotechnology and microbiological laboratory of the Central Organization for Standardization and Quality Control – Ministry of planning . Bacteria were grown on nutrient broth (Oxoid company) at 37°c for (24-48h) and then maintained on nutrient agar slants (Oxoid company) at 4°c. The extracts were dissolved in ethylene glycol, then filtered through membrane filter (0.45µm) sterilized and tested for antibacterial activity using disc diffusion method. 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 (2.5×10^8 cells /ml using pour plate method). The plates were incubated at 37^oc for 24-48 h. The experiments were carried out in triplicate. The results were recorded by measuring the zone of inhibition around the discs. Control disc contains ethylene glycol only was used for comparison. Standard antibiotics (gentamycin, ampicillin, erythromycin) inhibiting bacterial protein synthesis were included in the reseach. The antibacterial spectra showing zone of inhibition in millimeters and as percentage calculated in accordance to gentamycin as positive control with 100% inhibition. The extracts A (essential oil) B (chloroform) C (methanol) and D(water) were tasted for anti -bacteria activity.

Results and discussion:

Results of Table(1) and (2) show that all the extracts were found inactive against gram – positive *Staphylococcus aureus* and *Bacillus cereus* except extract–A(essential oil). Among gram– negative bacteria extract –A displayed moderate activity against *Salmonella* typhimurium and *Escherichia coli* while all other extracts were inactive. According to the results a bove minimum inhibitory concentration was studied only for essential oil (extract – A) and results were compared with standards antibiotic.

Table (1): Zone of inhibition for various extracts of *C.longa* compared with some antibioticts

Antibiotics	Tested microorganisms			
	Staphylococcus aureus **Zone of inhibition in mm**		***Bacillus cereus*** **Zone of inhibition in mm**	
	Mean	**percentage**	**Mean**	**percentage**
Gentamycin 30 micg	**28.24 ± 1.25**	**100**	**20.28 ± 0.92**	**100**
Ampicillin 10 micg	**25.44 ± 1.4**	**87**	**8.42 ±0.42**	**20**
Arthromycin 10 micg	**23.66 ± 0.72**	**79**	**6.00 ± 0.00**	**0.00**
Fraction-A	**13.10 ± 0.82**	**32**	**14.42 ± 0.82**	**59**
Fraction-B	**6.00 ±0.00**	**0**	**7.24 ± 0.62**	**9**
Fraction-C	**6.00 ± 0.00**	**0**	**6.74 ± 0.48**	**5**
Fraction-D	**10.24 ± 0.62**	**19**	**9028 ± 0.78**	**23**
Ethylene glycol	**6.00 ± 0.00**	**0**	**6.00 ± 0.00**	**0.00**

Mean value of diameter of inhibition zone with standard error as the diameter of paper disk used was 6mm,6mm 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 of *C.longa* compared with some antibioticts

Antibiotics	Tested microorganisms			
	Salmonella typhimurium Zone of inhibition in mm		*Escherichia coli*	
	Mean	percentage	Mean	Percentage
Gentamycin 30 mcy	32.14 ± 0.87	100	21.44 ± 0.24	100
Ampicillin 10 mcy	6.48 ± 0.20	2	13.26 ± 0.64	47
Erthromycin 10 mcy	6.00 ± 0.00	0	11.92 ± 0.36	38
Fraction – A	12.72 ± 0.64	29	8.22 ± 0.22	14
Fraction – B	6.00 ± 0.00	0	6.00 ± 0.00	0.00
Fraction – C	6.80 ± 0.24	4	6.60 ± 0.24	4
Fraction – D	7.24 ± 0.00	7	7.42 ± 0.26	9
Ethylene Glycol	6.00 ± 0.00	0	0.00	0.00

It was observed that dilution altered the activity gradually in gram– positive bacteria to 78% and 59% a gianst *Staphylococcus aureus* at 1/10 and 1/100 dilution respectively; however *B.cereus* did not show significant change at 90% at 1/10 dilution, although activity decreased to 66% at 1/100 dilution table (3) the gram – negative bacteria *S. typhimurium* and *E. coil* showed no activity below 400 µg / disc concentration(1/100 dilution).

Table(3) : Minimum inhibitory concentration of fraction – A(essential oil) on gram – positive bacteria with gentamycin as standard reference

Antibiotics	Tested microorganisms			
	Staphylococcus aureus Zone of inhibition in mm		*Bacillus cereus* Zone of inhibition in mm	
	Mean	percentage	Mean	percentage
Gentamycin 30 mcg	29.24 ± 0.66	100	21.22 ± 0.64	100
Fraction – A	15.84 ± 0.32	42	15.28 ± 0.42	61
1/10 of fraction A	13.72 ± 0.74	33	14.44 ± 0.32	55
1/100 of fraction A	11.82 ± 0.42	25	11.62 ± 0.32	36
Ethylene Glycol	00 ± 0.00	0.00	6.00 ± 0.00	0.00

The activity of the extract against *E. coli* was not affected at 1/10 dilution (decrease 87%) but it was at 1/100 (decrease 62%) table (4).

Table (4): Minimum inhibitory concentration of fraction –A (essential oil) on gram – negative bacteria with gentamycin as standard reference

Antibiotics	Tested microorganisms			
	Salmonella typhimurium Zone of inhibition in mm		*Escherichia coil* Zone of inhibition in mm	
	Mean	percentage	Mean	percentage
Gentamycin 30 mcy	22.45 ± 0.26	100	21 .65 ± 0.34	100
Fraction – A	11.28 ± 0.42	33	8.45 ± 0.22	16
1/10 dilution of fraction A	8.43 ± 0.32	14	8.12 ± 0.22	14
1/100 dilution of fraction A	6.22 ± 0.12	1	7.54 ± 0.42	10
Ethylene glycol	6.00	0.00	6.00	0.00

Essential oil extract is more effective against gram – positive compared to gram – negative *B. cereus* and *S. aureus* have shown 59 and 32% inhibition respectively compared to gram – negative strains *S. typhimurium* and *E. coli* showing of inhibition 29 and 14% respectively. Extract – A *(*essential oil) shows comparable activity to standard antibiotics. It has shown highest inhibition in gram – positive *B. cereus* (59%) and moderate activity in *S. aureus* (32%) compared to erythromycin, ampicillin and gentamycin, while extract – A showed significant activity (29%) against *S. typhimurium* compared to gintamycin table (2). The result is encouraging, as all other antibiotics were inactive against this bacteria. The present study suggest that essential oil extract from turmeric possesses significant antibacterial activity at very low concentration (40 µg /disc) on pathogenic gram – positive *B. cereus* and *S. aureus*. It has been suggested that the antibacterial effect of *C. longa* extracts is associated to the presence of hydroxyl and phenol groups in the molecule of turmeric beings essential for the inhibition of bacteria [7, 8, 15].

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