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ANTIMICROBIAL ACTIVITY AND CHEMICAL CONSTITUENTS OF DIFFERENT EXTRACTS OF RHIZOMES OF TURMERIC (*CURCUMA LONGA* L.) FROM WEST ANATOLIA

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ABSTRACT

To specitificate the chemical context and antimicrobial characteristic of rhizomes of turmeric (*Curcuma longa* L.) rhizomes extratcs obtained from Muğla, Turkey.The extract of ethyl acetate (EA), methanol(M) and water (W) of turmeric rhizomes were studied for antimicrobial characteristic against 11 bacteria and one yeast by disc diffusion method. Among the extracts assayed, the ethyl acetate extracts of turmeric rhizomes exhibited good activity against *B. subtilis* ATCC 6633, *B. cereus* CCM 99 at 100mg for example 20 mm was recorded as diameter zone of inhibition. The least inhibition zone of rhizomes is 9 mm against *K. pneumoniae* CCM 2318 at 50 mg. *C. albicans* ATCC 10239 showed no inhibition zone both 50 mg and 100mg doses. The M extracts of turmeric rhizomes displayed good activity against *S. aureus* ATCC 6538/P 100 mg for example 14 mm was recorded as diameter zone of inhibition. When we compared MIC value of the EA, M and W, the EA extract (MIC 1-16 mg/ml) was found to be very effective followed by the W extract (MIC 2-64 mg/ml). The least effective was the M extract (MIC 16-128< mg/ml). The W extracts presented the best activity (MIC 2 mg/ml) against *S. aureus* ATCC 6538/P compared with standart drugs.

KEYWORDS:
Curcuma longa L., turmeric, rhizomes, extract, antimicrobial activity, GC-MS

INTRODUCTION

Nowadays, usage of natural antibacterial compounds is take attention, herbs and spices for the preservation of foods, as these posses a characteristic flavour and sometimes show antioxidant activity and also antimicrobial characteristic [1]. To achieve this purpose, the food industry has used synthetic additives (added or already present naturally in the foods) which reduction microbial growth or inhibit microorganisms and prevent or delay, in a significant way. Howewer, even we discovered many antibiotic drug, we still facing multidrug resistance to bacteria [2,3] and the side effect of antibiotic treatment for patients like allergia. Some of the plant family have a antimicrobial activity like Zingiberaceae, Oleaceae, Liliaceae and Lamiaceae. Several plant parts are used for medicinal purposes ie., bulb, gel, leaves, roots, barks, peels etc. *Curcuma longa* L. (Family Zingiberaceae) is a a perennial herb with, pulpy, orange, tuberous roots that grows to abaout 2 feet in length and is cultivated extensively in India, China, Bangladesh and other Asian countries with a trpoical climate [4]. Curcumin has been reported to show anti-inflamatory, antioxidant and chemopreventive property [5]. And also, antimicrobial activity of *C. longa* have against various microorganisms [6-13].

Our paper is the first report on the antimicrobial activity of West Anatolian turmeric against pathogenic bacteria. Therefore, we have investigated for the antimicrobial activities of three different solvent extracts from West Anatolian turmeric. The aim of this study is to carry out a comparative analysis of the antimicrobial activity of extracts obtained from West Anatolian turmeric. For this reason, we have practiced antimicrobial effect against some microorganisms including opportunistic pathogens: Gram-negative bacteria; *Escherichia coli* O157:H7, *Escherichia coli* ATCC 35218, *Pseudomonas aeruginosa* ATCC 27853, *Salmonella typhimurium* CCM 583, *Aeromonas hydrophila* ATCC 19570, *Klebsiella pneumoniae* CCM 2318. Gram-positive bacteria; *Staphylococcus epidermidis* ATCC 12228, *Bacillus subtilis* ATCC 6633, *Bacillus cereus* CCM 99, *Staphylococcus aureus* ATCC 6538/P, *Streptococcus faecalis* ATCC 8043. Fungus; *Candida albicans* ATCC 10239.

Disk diffusion method and minimal inhibitory concentration (MIC) were used for antimicrobial activity of tested extracts.

MATERIALS AND METHODS

Sample collection and storage: turmeric preparations were obtained from various retail outlets in West Anatolia, including supermarkets, shops and market stalls in Cine, Turkey in 2011. Turmeric sample was stored at ambient temperature until initial sample preparation, after which they were stored at 4°C until required for analysis.

Preparation of *Curcuma longa* L. rhizomes extracts. Two 100 g portions of powder of dried rhizomes of *C. longa* were soaked separately in 1000 ml of ethyl acetate (EA) and methanol (M) for 72 h at room temperature. For water (W) extraction, 100 g of powdered sample were boiled in 1000 ml of hot water for 60 min [9].

Test microorganisms. The bacterial and fungal strains used for the screening were Gram-negative bacteria; *E. coli* O157:H7; *E. coli* ATCC 35218; *P. aeruginosa* ATCC 27853; *S. typhimurium* CCM 583; *A. hydrophila* ATCC 19570; *K.pneumoniae* CCM 2318. Gram-positive bacteria, *S.epidermidis* ATCC 12228, *B. subtilis* ATCC 6633, *B.cereus* CCM 99, *S.aureus* ATCC 6538/P, *S. faecalis* ATCC 8043. Fungus, *C. albicans* ATCC 10239.

Nutrient agar (NA) (Merck) and Potato-Dextrose Agar (PDA) (Merck) were used for testing the antibacterial and anticandidal activity.

Study of antimicrobial activity by disc diffusion assay. For the first screening, the paper disc diffusion method was used to determine antibacterial activity, which is based on the method described previously [14]. Tobramycin (10 µg/disc) (Oxoid), ampicillin (10 µg/disc) (Oxoid) and nystatin (30 µg/disc) (Oxoid) were used as positive controls and paper discs treated with ethyl acetate, methanol and DMSO were used as a negative control. The plates were then incubated at 35 °C for 24 h in an incubator. Inhibition zone diameters around each of the discs were measured and recorded at the end of the incubation time.

Determination of minimum inhibitory concentrations (MICs). MICs were determined by the agar dilution method, which is based on the method described previously [9]. The MICs of ampicillin (Oxoid) and oxacillin (Oxoid) were also determined. A final inoculum of 1×10^4 CFU/ml was spotted onto agar plates. The plates were then incubated at 35°C for 24 h in the incubator. The MIC was defined as the lowest concentration of extracts at which no visible growth was observed.

RESULTS AND DISCUSSION

In this work, the extracts of ethyl acetate, methanol and water of turmeric rhizomes were tested for antimicrobial activity against 11 bacteria and one yeast by disc diffusion method. The results are presented in Table 1. According to these data, the EA were the most active against tested bacteria both 50 mg and 100 mg doses. As compared with standart antibiotics. Among the extracts assayed, the EA of turmeric rhizomes exhibited good activity against *B. subtilis* ATCC 6633, *B. cereus* CCM 99 at 100mg for example 20 mm was recorded as diameter zone of inhibition. This followed by *S. aureus* ATCC 6538/P and *A. hydrophila* ATCC 19570 with 16 mm inhibition zone. The least inhibition zone of rhizomes is 9 mm against *K. pneumoniae* CCM 2318 at 50 mg. *C. albicans* ATCC 10239 showed no inhibition zone both 50 mg and 100mg doses.

TABLE 1
Antimicrobial activity of *Curcuma longa* L. rhizomes extracts against test microorganisms by disc diffusion method

Microorganism	Inhibition Zone (mm)											
	Ethyl acetate extract (mg)			Methanol extract (mg)			Water extract (mg)			Tobramycin	Ampicillin	Nystatin
	C	50	100	C	50	100	C	50	100	(10 µg/disc)	(10 µg/disc)	(30 µg/disc)
E. coli O157:H7	-	13	15	-	-	10	-	-	11	9	12	NT
E. coli ATCC 35218	-	11	14	-	-	10	-	-	10	10	12	NT
P. aeruginosa ATCC 27853	-	12	14	-	-	9	-	-	10	11	9	NT
S. epidermidis ATCC 12228	-	-	13	-	-	-	-	-	12	10	10	NT
B. subtilis ATCC 6633	-	17	20	-	9	12	-	-	15	15	10	NT
B. cereus CCM 99	-	15	20	-	8	13	-	-	16	16	12	NT
S. typhimurium CCM 583	-	11	13	-	-	-	-	-	12	10	12	NT
S. faecalis ATCC 8043	-	10	12	-	-	7	-	-	11	9	14	NT
A. hydrophila ATCC 19570	-	13	16	-	-	10	-	-	12	11	10	NT
K. pneumoniae CCM 2318	-	9	14	-	-	12	-	-	12	11	9	NT
S. aureus ATCC 6538/P	-	12	16	-	9	14	-	-	13	13	15	NT
C. albicans ATCC 10239	-	-	-	-	-	-	-	-	-	NT	NT	18

C, Control (only ethyl acetate, methanol or water); -, no inhibition; NT, not tested

TABLE 2
MICs of *Curcuma longa* L. rhizomes extracts, ampicillin and oxacillin against test microorganisms

Microorganism	MIC (mg/ml)				
	C. longa			Ampicillin	Oxacillin
	Ethyl acetate extract	Methanol extract	Water extract		
E. coli O157:H7	2	64	32	128	16
E. ATCC 35218	2	64	32	32	32
P. aeruginosa ATCC 27853	2	128	16	16	64
S. epidermidis ATCC 12228	16	128<	32	32	32
B. subtilis ATCC 6633	1	16	4	4	32
B. cereus CCM 99	1	16	4	4	16
S. typhimurium CCM 583	4	128<	8	16	16
S. faecalis ATCC 8043	8	128	64	64	4
A. hydrophila ATCC 19570	1	32	8	32	32
K. pneumoniae CCM 2318	2	32	4	16	128
S. aureus ATCC 6538/P	1	16	2	8	4

128<, no activity

The M extracts of turmeric rhizomes displayed good activity against *S. aureus* ATCC 6538/P 100 mg for example 14 mm was recorded as diameter zone of inhibition. This followed by 13 mm *B. cereus* CCM 99 at 100 mg. *S. epidermidis* ATCC 12228, *S. typhimurium* CCM 583 and *C. albicans* ATCC 10239 presented no inhibition zone both 50 mg and 100 mg. Our results were accordance with Gur and his friends [15] who reported that the M extracts of turmeric were the most effective on *S. aureus*.

While the W extract showed antibacterial activity against listed bacteria at 100 mg, none of the bacteria displayed antibacterial activity at 50 mg. The reason for these, curcumin is the product obtained by solvent extraction of turmeric i.e., the ground rhizomes of *Curcuma longa L.* and also curcumin is an oil-soluble pigment, practically insoluble in water [16]. All the extracts showed no anticandidal activity.

When we compared MIC value of the EA, M and W extract, the EA extract (MIC 1-16 mg/ml) was found to be very effective followed by the W extract (MIC 2-64 mg/ml). The least effective was the M extract (MIC 16-128< mg/ml). In contrast to, Aly and Gumgumjee (2011) [12]. tested antimicrobial activity of methanolic and butanolic extracts of *C. longa*. The diameter of inhibition zone ranged from 25 to 27 mm with mean antimicrobial index of 25 and from 14 to 24 mm with mean index of 19 mm for methanol and butanol *C. longa* extracts, respectively.

The EA extract displayed the best activity (MIC 1 mg/ml) against *B. subtilis* ATCC 6633, *A. hydrophila* ATCC 19570 and *S. aureus* ATCC 6538/P, *B. cereus* CCM 99. The W extracts presented the best activity (MIC 2 mg/ml) against *S. aureus* ATCC 6538/P compared with standart drugs. In a similarly, Niamsa and Sittiwet [17] reported that aqueous extract of *C. longa* exhibited antimicrobial activity against *K. pneumoniae* ATCC 10031, *Esherichia coli* ATCC 25922, *S. aureus* ATCC 25924, and *S. epidermidis* ATCC 12228 (MIC 4-16 gL^{-1}) and minimum bactericidal concentration (MBC) 16-32 gL^{-1}). And also, Chandrana and his friend [18] reported that antimicrobial activity of turmeric was effective against *E. coli, B. subtilis* and *S. aureus* and suggested that the activity is due to the presence of curcuminoid, a phenolic compound. In another similar work, Naz and his friends [19] reported that both curcuminoid and oil showed antibacterial activity against all tested microorganisms. Because of intrinsic tolerance of microorganisms change to varying degrees of sensitivity of the bacterial test organisms.

Pundir and Jain (2010) [13] reported that the ethanolic extract being strongly active against *E. coli* isolates while aqueous extracts strongly active against *S. aureus* isolates (25mm-30mm). The ethanolic extract of turmeric was effective in extraction of antimicrobially active substances as compared to water and hexane reported by Gur and his friend [16]. The ethanol extraction of herbs and spices was better because ethanol is an organic solvent and dissolves more organic compounds. Therefore, the greater amounts of active antimicrobial components have been acquired [20]. Irhsad and his friends [21] reported that the ethanol extract of turmeric displayed the highest zone of inhibition (11 mm) against *B. subtilis* followed by *E. coli* and *S. aureus* that were 10 mm and 9 mm, respectively. The methanol extract of turmeric displayed maximum activity against *B. subtilis* (9 mm) followed by 8 mm for *E. coli* and *S. aureus.* And also, Mirbod and his friends [22] reported that feeding incremental levels of CRP(*C. longa* rhizome Powder) decreased *E. coli* enumeration in the ileal content of laying hens. No and his friends [23] reported that the curcumin nanoparticles (CNPs) formulated with positively charged surfactant cetrimonium bromide (CTAB) exhibited the highest antimicrobial activity against *L. monocytogenes*, indicating that there is a strong relationship between surface charge and antimicrobial activity of curcumin. The enhanced

antimicrobial action of CNPs-CTAB was concluded to be due to the increased cell-antimicrobial interaction, which resulted from the opposing electrical charges between CNPs-CTAB and *L. monocytogenes* cells, as well as increased antimicrobial penetration endowed by the small size. One study about phytochemical composition of turmeric, Zhang and his friends reported that [23] 5% and 10%, are *β*-sesquiphellandrene, aromadendreneoxide and germacrone for *C. longa* main components.

Conclusion, all the extracts of rhizomes of turmeric showed varying degrees of antimicrobial activity on the microorganisms tested. Some of these extracts were more effective than conventional antibiotics to combat the pathogenic bacteria and fungus tested. Among the tested extracts the EA extracts were the most active against the microorganisms tested compared to the antibiotic standart. All the extracts showed no anticandidal activity. So, pharmacological test is essential to isolate and characterize their effective compounds. Moreover, these plants extract should be researched *in vivo* to better understand their safety, activity and properties.

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