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Turmeric: Alternative Therapy Against MDR *Staphylococcus aureus*, Preservative, Shelf-life the Miced Meat.

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Abstract:

Aqeous and alcoholic extract of turmeric (*Curcuma longa)* were evaluated for its antimicrobial action against the food borne and food spoilage pathogen, *Staphylococcus aureus,* that resistant concentrations (Minimum inhibition concentration (MIC) and Minimum bactericidal concentration (MBC)) of *C.longa* against *S.aureus* were found using MIC and MBC assay,miced meat was taken as the standard sample to test for the turmeric upon MDR *S. aureus*during 6 days storage and compared with sodium nitrate.Turmeric extract exhibited anti-MDR *S.aureus* activity with all concentration-dependent relationship.the alcohol extracts were the most efficient,then aqueous extracts and both of them with high significant differences compared with sodium nitrate(preservative). The value of MIC and MBC of turmeric was 2.5 ml and was the efficient when used in miced meat storage assay for 6 days with high significant differences compared with sodium nitrate.

الخلاصة:

تم تقييم الفعالية المضادة للجراثيم لمستخلصي مسحوق جذور الكركم المائي والكحولي ضد بكتريا المكورات العنقودية الذهبية *Staphylococcus aureus*/ *لمقاومة* للعديد من الادوية والمسببة لتلف الاغذية باستخدام طريقة الانتشار في الحفر، وتم ايجاد التركيزالادنى المثبط MIC والقاتل MBC للمسحوق ضد *S.aureus*.

اخذ اللحم المثروم كنموذج قياسي لاختبارمسحوق الكركم ضد MDR*S.aureus* وخلال 6 أيام خزن وقورنت مع الصوديوم نتريت.

بينت النتائج ان لمستخلص الكركم فعاليه ضد MDR*Saureus* ولجميع التراكيز ذات العلاقة,المستخلص الكحولي كان اكثر كفاءة ثم المائي وكلاهما بفروق معنوية عالية مقارنة بالصوديوم نتريت (الماده الحافظة). قيم MIC وMBC للمسحوق كانت 2.5مل وكانت الاكفأ حين استخدمت في طريقة خزن اللحم المثروم لمدة 6 أيام وبفروق معنوية عالية مقارنة بالصوديوم نتريت.

Introduction:

Many types of fresh food products are prone to rapid microbiological spoilage because it supports the growth of microorganisms[1]. Therefore, synthetic additives have been used in the food industry to inhibit microbial growth but the consumer concern about the harm associated with synthetic additives (14). Natural additives from herbs are interesting to be used in food products. Consequently, search for natural additives has notably increased in recent years.Several researchers have reported a potential of spice and herb extracts as an antimicrobial agents to prevent it in food product[2,3]. One of these spice is a Turmeric which obtained from rhizomes of plant *Curcuma longa,* a member of the family Zingaberaceae, components of turmeric are named curcumnoids, which include mainly curcumin (diferuloyl methane), demethoxycurcumin and bisdeme thoxycurcumin[4]. Previous studies showed the ethanol extract of turmeric exhibited the strongest inhibitory effect against *Staphylococcus aureus* as medicinal plants[5]. *S. aureus is* the bacteria that causes Staph infection and afrequent culprit in cases of food poisoning, generally due to improper food handling and inadequate hygiene by produce a toxine that causes the illness[6]. So these extracts may be an alternative to chemical preservatives and used as natural antimicrobial preservatives to reclaime the shelf-life of food[7]. Development of *S.aureus* resistance to the available antibiotic and increasing popularity of traditional medicine had led researchers to investigate the anti *S.aureus* compound in plants[15]. Turmeric is commonly useful and have a wide spectrum of biological action,so became one of the methods to reduce the antibiotic resistance because it is considered

safe (people are able to take up 10 grams per day for a period of a few weeks without notice problems,there is no significant toxicity and it is often formulated with brome lain for increased absorption and enhanced anti-inflammatory effect[9,10.11]. Allthese causes (demanded for wholesome, antibacterial, give a flavor, color and enhance aroma of food, enhance the medical values of food, safe and reclaime the shelf-life of food, no side effect ,antioxidant and anti-inflammation enable us to say: The aim of this study is to lay a theoretical foundation for development its natural presservative, alternative therapy.

Materials and Methods:

1- Plant material: The rhizome of *C.longa* were collected from local market (Al-Shourja), the sample was identified by qualified Taxonomist.

2- Preparation of turmeric rhizome: Rhizomes were cleaned and air dried for 2days, the dried sample was again dried in a hot air oven at 50^{o}C for 24 hrs, then ground into powder and pass a sieve with nominal mesh size of 2mm in diameter.

3- Turmeric extraction: Turmeric powder was extracted with ethanol 70% and distills water (each of combined filtrate was concentrated under vacuum at 35^{o}C even drying then put in dark vial in freeze. For working made stock solution (100mg powder extract ×10ml D.W) 4 concentration from stock solution were taken (25, 50, 75, 100) mg/ml to prepare the concentration (2.5, 5, 7.5, 10) ml for antibacterial activity against *S.aureus* and compared with sodium nitrate.

4- Chemical preservative: The preservative used in the experiment was: sodium nitrate (SN), with a concentration of 3 mg/ml from D.W.

5- Test bacteria and source: Five isolates of *S.aureus* were used. Three (S1, S2, S3) were obtained from Central Health Laboratories in Baghdad and two (S4,S5) from High studies laboratory in collage of science, AL-Mustansiriyah University, all these isolates were identified by the chemical test, The multi-drug resistant isolate was chosen in the residue tests of study. The stock culture was kept in refrigerator (4^{o}C) on nutrient agar.

6- Preparation of bacterial suspention: Respective suitable slant medium was used to activate the *S.aureus* isolates to be tested by means of sterile operation and inoculate it's into the corresponding liquid medium, it was taken as stock solution after culturing in the constant temperature incubator at the most suitable temperature for 16-18 hrs. Bacterial suspensions containing bacteria of about 10^{6} cell/ml was prepared with sterile physiological saline for further use.

7- Antibiotic used: 10 types of antibiotic were used in this study to know the more resistant *S.aureus* isolate to drug.

8- *S.aureus* sensitivity test to used antibiotic: by using Muller Hinton Agar (MHA), *S.aureus* isolates were tested, 0.1 ml of these cultured isolates were spreaded on MHA medium and with sterile forcipes the antibiotic disc were transferred, incubated with 37^{o}C for 24 hrs. Result was read by measurement diameter inhibition zone (millimeter) around antibiotic disc. Then, the more resistant isolate to all antibiotic discs was chosen for following tests and named MDR *S.aureus.*

9- Agar well diffusion method: The antibacterial activity of crude extracts (aqueus, ethanolic) of turmeric against the MDR *S.aureus* isolate was evaluated by using Agar Well Diffusion method[19]. 0.1ml of bacterial suspension was spreaded with sterile swabs on MH plate's agar; wells of 2mm size were made with sterile borer. 0.1 ml of the turmeric extracts (4 concentration of each extract type) was poured into a well of inoculated plates, the plates thus prepared were incubated for 24 hrs at 37^{o}C, after incubation, it was indicated by an inhibition zone surrounding the well containing the turmeric extract, the diameter inhibition zone was measured and expressed in millimeter (mm).

10-Determination of minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC): The crude extracts were prepared at concentrations of (2.5, 5, 7.5, 10) mg/ml in distilled water, the MIC and MBC were determined by a broth dilution method[15]. Nutrient broth samples (10ml) were inoculated with the different concentrations of the aqueous and alcoholic extract (each of them alone) and with 0.1 ml of active inoculums of MDR *S.aureus* (approximately 10^6 cell/ml) for 24 hrs at 37^{o}C, the viable plate counts determined by spreading a 0.1ml sample of each treatment on the surface of Nutrient Agar (NA) and the colonies were counted after incubation. The MIC and MBC was defined as the minimum level of the extract that produced a 90% reduction in growth of the MDR *S.aureus*.MBC was the lowest concentration that killed at least 99.9% of the initial inoculums.

11-Effect the turmeric extract upon MDR *S.aureus* during 6 days storage in miced meat: 150 gm Part (1): miced meat without addition of neither turmeric nor sodium nitrate, just MDR *S.aureus* isolate as control for panel test. Part (2): miced meat with MDR *S.aureus* isolate and sodium nitrate without addition turmeric as control for testing the growth with chemical preservative. Part (3): miced meat with turmeric and MDR *S.aureus* isolate as control for testing the growth with natural preservative. NOTICE:

1. The initial count for MDR *S.aureus* isolate was 10^6 cell /ml.
2. The MIC and MBC of turmeric powder were added to miced meat inoculated with MDR *S.aureus.*

12-Statistical analysis: Data are expressed as Mean±SEM of triplicates. Students t-test was used to compare the anti MDR *S.aureus* by the extracts (between them) and the chemical preservative sodium nitrate. All statistical analysis was conducted with SPSS software at significant levels of 0.05, 0.01, and 0.001.

Result and Discussion:

In this study, the isolate (S3) showed the complete resistance to all antibiotic used, then (S2) was the merest resistance among the residue, it was resistant to (7) antibiotic (GN, ER, AX, PY, ATM, IPM, KF), whereas the resistant was reduced in each of (S1, S4, S5) to (4, 4, 5) antibiotic respectively.

The resistance and sensitivity that showed by *S.aureus* are explained by many options like: the nature of bacteria itself, physiological structure, genetic, mutation, etc. we will not discuss these options because this is not our objective; we chose the (S3) and named MDR *S.aureus* (Table-1).

Table-1: *S.aureus* isolates resistant to Antibiotic

Antibiotics	symbol	*S.aureus* isolates				
		S1	S2	S3	S4	S5
Gentamycin	GN	R	R	R	R	S
Doxycyclin	DOX	S	S	R	S	S
Erythromycin	ER	R	R	R	R	R
Amoxicillin	AMX	S	R	R	R	R
Methicillin	MTN	S	S	R	S	S
Ciprofloxacin	CIP	S	S	R	S	S
Carbencillin	PY	R	R	R	R	R
Aztreonam	ATM	S	R	R	S	S
Impenean	IPM	S	R	R	S	S
Cephaithin	KF	R	R	R	S	R

R: resistant, S: sensitive

Figure-1show the turmeric extracts were displayed effective antimicrobial activity (with all concentration) against food spoilage, food borne MDR *S.aureus*, compared with chemical preservative sodium nitrate (SN). The zone of inhibition the extracts generally against sensitive MDR *S.aureus* was in the range of (5-22) mm, alcoholic extract was the best (8-22) mm compared with aqeous extract was just (5-15) mm and SN was (4) mm only with standard concentration that allowed in world. The alcohol extract of turmeric showed high significant differences ($p<0.001$) zone of inhibition against MDR *S.aureus* compared with water (aqueous) extract that showed moderate inhibition ($p<0.01$), whereas SN exhibited less (and lower) inhibition ($p<0.05$) against MDR *S.aureus*.

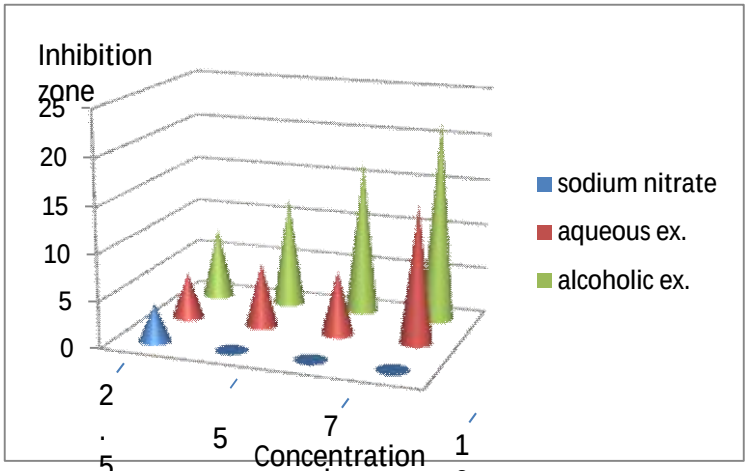

Figure-1: Anti-MDR *S.aureus*activity of turmeric extract (aqeous and alcoholic)and sodium nitrate by agar well diffusion.

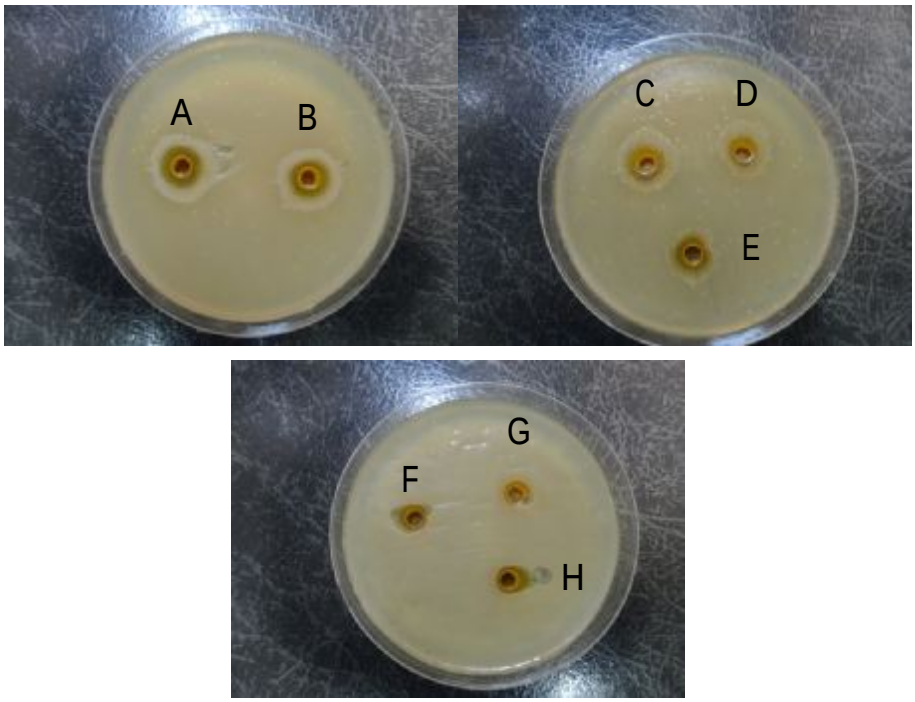

Figure-2: Anti MDR *S.aureus* activity of turmeric extract and sodium nitrate by agar well diffusion. A, B, C: alcoholic ex. Conc. (5, 7.5, 2.5) mg/ml respectively. F, H, D, E: aqueous ex. Conc. (2.5, 5, 7.5, 10) mg/ml respectively. G: sodium nitrate conc. (3ml).

These results are in parallel with the findings of previously reported studies that alcohol extract is the better solvent for consistent extraction of anti–MDR *S.aureus* components from turmeric compared to other solvents, the alcohol extract of any plant (generally) and spices like turmeric (especially) was better because of alcohol (ethanol or methanol) is an organic solvent and dissolves more organic compounds ,resulting in the liberation of the greater amounts of active antimicrobial compo-nents[12,21]. One of these components is curcumnoids: turmeric consists of 3-5% curcumnoids, it is the most important fraction which is responsible for the biological activities of turmeric ,it is insoluble in water but soluble in alcohol (13), these results were the same results of our present study [13,16].

Also, in other studies we can find some authors that associate the antimicrobial activity of turmeric to the presence of curcumnoids and its derivatives from the hydroxyl and phenol groups in the molecule and the phytochemical seems to be a potential source, having different substances to be investigated, including alkaloids, flavonoids curcumin, trepenoids, etc. which have efficacy against bacteria generally and *S,aureus* especially[13,8].

Generally, the high concentration of phenolic compounds in turmeric accounts for their antioxidant property and the enormous scientific studies reported by several researchers on the anti *S. aureus* activities of turmeric, so this anti- microbial may be due to the high positive correlation between anti *S.aureus* activity, total phenolic content and antioxidant property caused by curcumin (or curcumnoids) of turmeric[19,20]. In addition to that, turmeric is known to contain another active ingredients, ptolymethyl, carbinol and essential oils which may be responsible for its anti-*S.aureus* activities[16].

At last, curcumnoids are compounds that protect the body's cell from damage caused by activated oxygen molecule known as free radicals, these mechanisms called antioxidant property[12], and in vitro, this anti *S.aureus* may be attributed to the presence free radical scavenging (ROS) in both turmeric extract in vitro, ROS scavenging ability and cell proliferation in cell line that inhibited the growth of activity of MDR *S.aureus* have shown in Senguputa et al, (2011) works, when we compared these findings with the chemicals preservative sodium nitrate: *in vivo*, it posses very dangerous side effect causes cancer disease in future[14]. *in vitro*, in our study it didn't inhibit the MDR *S.aureus* just (4mm) the less and lower inhibition zone, and this means SN have weak ability to inhibition, several studies have reported these rules[18,20].

The MIC and MBC value varied depending on the concentration of the extract. Results are represented in Table (2) that shows there is a wide range of MIC and MBC values, an alcohol extract of the turmeric showed the highest anti MDR *S.aureus* activity with an range of (2.5-3) ml., the MIC of the turmeric aqueous extract ranged from (5-7.5)ml and the results for the MBC values were similar to the MIC values .the concentration 2.5ml (MIC of turmeric alcohol extract)was taken with equal as powder (25mg/ml) to show its activity as natural preservative in the follow test (limition total MDR *S.aureus* count).

Table-2: Minimum Inhibition Concentration (MIC) and Minimum Bacteriocidal Concentration (MBC) of turmeric extract (alcohol and aqeous) against MDR *S.aureus.*

Parameters (ml)	Turmeric extract	
	Ethanol	Aqueous
MIC	2.5	5
MBC	2.5	5

The mechanisms of anti *S.aureus* action of turmeric and derivatives is not yet clear, but hypothesis have been proposed different workers which involve: hydro phobic and hydrogen bonding of phenolic compounds to membrane proteins, followed by partition in the lipid bilayer, perturbation of membrane permeability consequent to its expansion and increased fluidity causing the inhibition of membrane embedded enzymes, membrane disruption, destruction of electrons transport systems and cell wall perturb bation[21].

Data presented in Table(3): showed that, total MDR *S.aureus* count were ranged from 2.97×10^6 to 3.11×10^7 at the end of storage period for control miced meat, meanwhile in the miced meat treated with turmeric gave a count of 3.28×10^2 and not detected at the end of storage period and showed high significant differences ($p<0.001$) compared with miced meat treated with chemical preservative SN that reached in count 3.11×10^7 until the end of the storage period which revealed the moderate significant differences ($p<0.01$), therefore, the results of presents study were agreed with many studies[22,23].

Table-3: Effect turmeric and sodium nitrate upon total MDR *S.aureus* count during miced meet storage period.

Storage period/days	Total MDR *S. aureus* count in miced meat		
	A	B	C
0	10^8	10^8+4ml	10^8+25mg
1	$10^6\times2.97$	$10^5\times5.20$	$10^2\times3.28$
3	$10^6\times3.25$	$10^6\times3.15$	10×2.79
6	$10^7\times3.11$	$10^7\times2.37$	-

A: Control micedmeat, 10^8 MDR*S.aureus*without adding anything.
B: Miced meat with 10^8MDR *S.aureus*+sodium nitrate (standared concentration).
C: Miced meat with 10^8 MDR *S.aureus*+turmeric (MIC).
0 Day: preparation all these concentration before incubation.
1, 3, 6 days: after incubation 3^o7C.

We remembered many reasons for this activity above, but there is another like: the inhibitory activity causative of turmeric constituents against *S.aureus*may be appear by biologically active of this components against sortase A, a bacterial surface protein anchoring transpeptidase from *S.aureus*, from these components is curcumin ,in addition to it was exhibited potent inhibitory activity against *S.aureus*, it is also a potent inhibitor of sortase A[25].Cell adhesion to fibronectin, the suppression of fibronectin-binding activity by curcumin highlights its potential for the treatment of *S.aureus* infections via inhibition of sortaseactivity. These results indicate that curcumin is a possible candidate in the development of bacterial sortase A inhibitor[11]. Because of turmeric showing target sites other than those used by antibiotics, so, if the presence of anti–MDR*S.aureus* substances in the turmeric is well established, turmeric have provided as source of inspiration for novel drug compounds as plants derived medicines have made significant contribution towards human health[22,24]. So it is expected it will be very active against drug resistant *S.aureus*, or like: the high anti-staphylococcus activity may be belonged to the active constituents of turmeric (in addition to the components were mentioned in the beginning)which are: the flavonoid curcumin (diferuloylmethane) and various volatile oils, including tumerone, atlantone and zingiberone, other constituents include sugars, proteins and resins, so the potent activity may be due to action all of these components together[8]. At last, turmeric

effect on Staphylococcus bacteria was agreed with many studies[24,25,26].

The best of our knowledge this is the first study of the using turmeric in two parts: first, as preservative, shelf life the food, inhibition pathogenic and spoilage food bacteria, second, as drug (alternative therapy) through its ability to killing multi-drug resistant *S.aureus* rather than antibiotics.

Conclusion: Considering the results, it may be concluded that, turmeric tested in the performed experimental conditions, may successfully inhibit MDR*S.aureus in vitro* as safe levels for human consumption and consequently, it can be useful as natural preserver or unspecific anti-MDR*S.aureus* food preserver.

And we reported here, the novel function of turmeric as an anti MDR *S.aureus* agent showing its higher activity than antibiotics, it can be used in controlling number of this resistant bacteria in food and treatment *S.aureus* infection.

Recommendation:

we invite researchers to investigate new curcuminoids derivatives with chemical modification based in structure and biological activity relationships ,in order to find, first: biopreservative, shelf–life the food, inhibit food spoilage food microorganisms; second: new drugs that can be without toxicity to humans and also can be used for the treatment of many disease generally and *S.aureus* infections especially. So, it is used for commercialization in the form of food preservative, additives, nutraceutical foods and drugs.

Finally,we hope that phytotherapies in official health care signal a new cycle of natural products research.

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