SCREENING THE ANTIBACTERIAL POTENCY OF *Curcuma longa* L. ESSENTIAL OIL EXTRACT AGAINST BOILS CAUSING *Staphylococcus* SPECIES

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ABSTRACT

Thirty five samples were collected from boils infections of teaching staff members and students of Biology Department / College of Science / Al-Mustansiriyah University / Baghdad /Iraq. Twenty isolates were identified as follows: ten *Staphylococcus aureus* and ten *Staphylococcus epidermidis* by bacteriological tests. Essential oil from the rhizome of turmeric (*Curcuma longa* L.; ginger family: Zingiberaceae) was extracted by hydrodistillation. The concentrated freshly extracted oil (100%) was used, as well as three other concentrations (50%), (33.3%) and (25%) were prepared. The present study was conducted to investigate the antibacterial effects of oil extract concentrations on all the twenty bacterial isolates by using both well diffusion (WD) and disc diffusion (DD) assays. According to the results, the concentrated oil (100%) had the best inhibitory activity against the isolates among the tested concentrations of oil. It could inhibit all the isolates of *S. aureus* by using both of the two methods, while all *S. epidermidis* isolates were inhibited by WD method only. In addition, this concentration showed maximum diameters means of inhibition zones (DMIZ) which were measured as (12.6 and 16.5) mm against *S. aureus* and *S. epidermidis* respectively. Results of using WD method revealed significant differences between concentrations (33.3%) and (25%). In contrast, the same concentration (100%) exhibited minimum DMIZ against *S. aureus* (9.5 mm) and *S. epidermidis* (8.3mm) and shows significant differences with only (25%) concentration by DD method. In conclusion, turmeric rhizome oil extract have an antibacterial activity against boils causing *Staphylococcus* species and its potency elevated by increasing the oil concentration. Furthermore, WD was the best method for determining this activity.

KEYWORDS: Antibacterial potency, *Curcuma longa* L., *Staphylococcus aureus,* hydrodistillation

INTRODUCTION

Infectious diseases still represent an important cause of morbidity and mortality among humans, especially in developing countries (Nascimento *et al*., 2000; Betoni *et al*., 2006). Boils is a very common skin problem around the world and the victim of this infection can belong to any age group. Mostly adolescents and children are easy victims of boils. A boil, is an infection involving the entire hair follicle and the surrounding skin. It is also called furuncle (Habif, 2009). A range of microorganisms are found on normal human skin, some of which are present as part of the resident commensal flora. On normal undamaged skin these microorganisms are considered nonpathogenic. However, under certain conditions, they cause opportunistic infections that may present serious medical problems (Holland, 2008). *Stapylococcus* is normally resides on the skin, usually not causing problems until a cut or other injury allows it to enter the protected tissue resulted in the production of boils. *S. aureus* continues to be one of the most difficult pathogens to treat because of its resistance to antibiotics. The most resistant strains have typically been found in hospitals, particularly in intensive care units, where antibiotics are extensively used (Guilfoile, 2007). With the continuous use of antibiotics, there is an increasing resistance of microorganisms towards them. In addition to this problem, antibiotics are sometimes associated with adverse effects on host which include: hypersensitivity, depletion of beneficial gut and mucosal microorganisms, gastric disturbances, ototoxicity, nephrotoxicity, immunosuppression and allergic reaction (Lopez *et al.*, 2001; Cosgrove *et al.*, 2009). Due to the above reasons of using synthetic antibiotics and because there is a constant need for new and effective therapeutic agents (Bhavnani and Ballow, 2000; Singh and Jain, 2011), many researchers have focused on the investigation of an alternative antimicrobial drugs from natural products as a source of new bioactive molecules for treatment of infections (Cordell, 2000;

Singh and Jain, 2011). In an attempt to improve the quality of life, plants have been used as a source of food, clothing, medicine, cosmetics and for seeking relief from hardship of life. Some plants are known as medicinal because they contain active substances that cause certain reactions; from relenting to the cure of diseases; on the human organism (Silva *et al*., 1994). Many medicinal plants produce antioxidant and antimicrobial properties which protect the host from cellular oxidation reactions and other pathogens (Wojdylo *et al*., 2007). There are many advantages in using antimicrobials obtained from medicinal plants such as fewer side effects as compared to commercial antibiotics, relatively less expensive, better patient tolerance, acceptance due to long history of use and being renewable in nature (Kim, 2005; Prabu *et al*., 2006). Spices and herbs have been used for thousand of the centuries by many cultures to enhance the flavour and aroma of food. Bioactive phytochemical constituents like alkaloids, phenolics, flavonoids, tannins, saponins and essential oils are usually responsible for medicinal importance of herbal plants (Krishnaiah *et al*., 2009). Essential oils are one of the most valuable natural products with multiple pharmacological activities. Essential oils from different spices were found to possess antimicrobial activity (Ozcan *et al*., 2006) and majority of the essential oils are classified by FDA as 'Generally Recognized as Safe' (GRAS) (Kabara, 1991; Parveen *et al*., 2013). An attempt was made to improve the quality of street foods by adding of plant essential oils into these foods as natural preservatives (Rath and Patra, 2012). This has recently attracted the attention of many scientists and encouraged them to screen plants to study the biological activities of their oils from chemical and pharmacological investigations to therapeutic aspects (Lahlou, 2004). Earlier studies have suggested that several essential oils have therapeutic potential, mainly in diseases involving skin infections. (Lawerence, 2005; Lopes-Lutz *et al*., 2008). *Curcuma longa*, better known as turmeric, is a

medicinal plant that botanically related to the ginger family: Zingiberaceae (Chattopadhyay, 2004). It is originated naturally in India and now is cultivated in tropical and subtropical regions of the world (McCaleb *et al*., 2000). Turmeric widely used as a spice, food preservative and coloring material in Asia and Middle East. It has been thousands of years that this golden colored spice is used in Indian system of holistic medicine, known as Ayurveda, and other traditional medicine specially as a treatment for various respiratory condition and also other diseases like liver disorders, anorexia, parasitic infections, rheumatism, diabetic wounds, sinusitis, cold and flu symptoms (Goel *et al*., 2008). There are several data indicating a great variety of pharmacological activities of turmeric, which exhibit anti-inflammatory, anti-human immunodeficiency virus (HIV), anti-bacteria, antioxidant effects and nematocidal activities (Loen and Araujo, 2001) and its use for the treatment of tumors (Sharma *et al*,. 2005). Other of its documented medicinal uses included that external application of turmeric stops pain and swelling, heals wounds rapidly, and treats many skin diseases ranging from acne to leprosy (Biswas, 2003). Turmeric paste is applied to the skin of the bride and groom before marriage in some parts of India, Bangladesh, and Pakistan, where it is believed to make the skin glow and keep harmful bacteria away from the body (Prasad and Aggarwal, 2011).

Turmeric contains phenolic compounds called curcuminoids that are responsible for the yellow color of the plant, and particularly of its rhizome. In fact, it is the curcuminoids that possess all the bio-protective properties of turmeric. Three main curcuminoids were isolated from turmeric: curcumin (C), demethoxycurcumin (DMC) and bisdemethoxycurcumin (BDMC) (Majeed *et al*., 2004). Curcumin, a natural polyphenolic flavonoid isolated from the rhizome of a plant has been found to possess many beneficial biological activities (Mun *et al*., 2013). More than 100 components have been isolated from turmeric. The main component of the root is a volatile oil which is responsible for the aroma of turmeric. These oils are d- -phellandrene, d-sabinene, cinol, borneol, zingiberene, sesquiterpenes, turmerone, arturmerone (Ohshiro *et al*., 1990; Kirtikar and Basu, 1993; Ruby *et al*., 1995). Volatile oil isolated from *C. longa* had been reported to have antibacterial (Norajit *et al*., 2007) and antifungal activities (Behura *et al*., 2000). Rath *et al* (2001) found that turmeric rhizome oil exhibit antibacterial activity against *Escherichia coli*. Burt (2004) also observed that the essential oil of turmeric could inhibit *Bacillus cereus* growth. On the other side, Babu *et al*., (2007) have published their work denoting the antifungal activity of turmeric rhizome oil against species responsible for causing infections.

Application of medicinal plants as alternatives to chemical agents for the treatment of infections has been reported by many authors (Prabuseenivasan *et al*., 2006), but few researchers focus on the antibacterial action of their natural products; especially the effectiveness of essential oils; against skin infections. Therefore, this study aimed to investigate the antibacterial potency of *C. longa* L. rhizome oil on *Staphylococcus* species isolated from boils infections and focused on determination of the most effective assay used for detection of antibacterial activity of this oil.

MATERIALS AND METHODS
Pathogenic Samples Collection
Thirty five boils swabs were collected from different patients who were teaching staff members and students of Biology Department / College of Science / Al-Mustansiriyah University / Baghdad / Iraq. Patients were 15 females and 20 males and their ages ranged from 18-40 years old.

Bacterial Isolates Identification
Isolation and Identification of boil causative agents were performed according to the methods described by Forbes *et al*.,

(2007) and Brown, (2011). The samples were collected using sterile swabs and inoculated on Mannitol Salt Agar and Blood Agar for isolation of *Staphylococcus* species. Coagulase test is considered an excellent definitive test for confirming identification of *Staphylococcus aureus* from Staphylococcus *epidermidis* isolates.

Bacterial Suspension Preparation (Test Microorganisms)
The bacterial species used in this study were two Gram-positive bacteria: *Stapylococcus aureus* and *Staphylococcus epidermidis.* Bacterial Suspensions were prepared on the day of work by transferring a minimum of two colonies from the overnight bacterial culture into 2 ml of distilled water.

Plant Materials
Rhizomes (underground stem) of *Curcuma longa* were purchased (obtained) from local traditional markets in Baghdad. The sample was identified by qualified taxonomist. It was washed with tap water to remove soil followed by sterile distilled water. *C. longa* rhizomes were grounded to powder with help of electric grinder and the powdered material was used immediately to extract the essential oil (Lin *et al*., 2004)

Essential Oil Extraction
Essential oil of the plant was extracted by submitting the powder of the grounded rhizomes for three hours to hydrodistillation using a Clevenger- apparatus according to the method recommended in the European Pharmacopoeia (2004) and as described by Kim *et al*., (2008). The extracted essential oil was yellow-greenish spicy liquid in appearance which was kept at 4ºC in Sterile dark brown bottle until use.

Oil Concentrations Preparation
After the extraction by a Clevenger-apparatus, the obtained oil was considered as (100%) concentration. Other concentrations were prepared by mixing a definite amount of concentrated oil with different volumes of ethylene glycol (1:1, 1:2, 1:3) to attain the concentrations: (50%), (33.3%) and (25%) respectively. Each concentration was then sterilized by passing through 0.45 µm Whatman no.1 filter paper, and the filtrates were stored at 4ºC until using for screening the antibacterial activity.

Antibacterial Activity Screening of the Essential Oil
1) Well Diffusion Assay (WD)
 Muller-Hinton Agar (MHA) plates were prepared. Each plate was inoculated with 100µl of bacterial suspension of each isolate (in duplicate) and spread with a sterile cotton swabs over the entire surface of the plate. The plates were allowed to dry for at least 15 min. Wells of 5mm diameter were made with sterile borer on MHA plates containing the bacterial inoculums. 100µl volume of known oil concentration was filled into a well of inoculated plates. The solvent (Ethylene glycol) was used as a control which was introduced into a well instead of plant oil. The plates were left at room temperature for ten minutes allowing the diffusion of the oil into the agar. After incubation at 37ºC for 18-24 hrs, the plates were observed for inhibition zones surrounding the wells. The diameters of inhibition zones (DIZ) were measured and expressed in millimeters (mm). Each oil concentration was conducted in duplicate in order to calculate the mean value (Rios *et al*., 1988)

2) Disc Diffusion Assay (DD)
A set of sterile filter paper discs of 5 mm diameter using Whatman no. 1 were prepared. The test microorganisms were transferred from freshly prepared suspensions to Muller Hinton agar (MHA) plates with the help of sterile cotton swabs. Each filter paper disc was loaded with 50 µl of a known oil concentration. A set of discs each loaded with the same amount (50 µl) of ethylene glycol were prepared for using them as control discs. All the discs were dried under laminar air flow cabinet. Using a sterile forceps, the discs were aseptically placed over the MHA plates previously seeded with the test bacterial suspensions. Plates were incubated in an upright position at 37ºC for 18-24 hrs and observed for distinct, clear zones of inhibition

around the discs which indicated positive microbial activity of the oil and the diameters of inhibition zones (DIZ) were determined. All the experiments were carried out in duplicate (Rath *et al*., 2005; Talaro, 2008).

Statistical analysis

The data (inhibition zones diameters) were expressed as mean ± standard deviation (S.D.). One way analysis of variance (ANOVA) was used and t test was used for the comparison between oil's concentrations for each bacterial species. Statistical analysis and significance, as measured by the above tests were performed using SPSS system. In all comparisons, P 0.01, P 0.05, P 0.05 were considered statistically highly significant, significant and not significant, respectively.

RESULTS

From the total of thirty five collected boils swabs samples, twenty isolates were obtained: ten isolates were *S. aureus* and the other ten isolates were *S. epidermidis*. The antibacterial activity of different concentrations of *C.longa* rhizome oil was evaluated against all the twenty bacterial isolates by using well diffusion and disc diffusion methods. All the concentrations used in research study were able to inhibit most of the bacterial isolates, but not all the tested isolates were sensitive to the examined concentrations. Table (1) shows the numbers of sensitive isolates of *S.aureus* distributing according to the examined oil's concentrations and tested methods. The best activity was shown by 100% conc. which had the ability to inhibit all the ten isolates when it was examined by both of the two methods. So this concentration could inhibit the highest number of the isolates, while the lowest number of the sensitive isolates was when 33.3% and 25% concentrations were tested by WD method.

TABLE1: Numbers of *S.aureus* sensitive isolates against *C.longa* oil's concentrations by WD and DD methods.

Method	Oil's Concentrations			
	100%	50%	33.3%	25%
WD	10	9	6	6
DD	10	9	9	7

WD = Well Diffusion   DD = Disc Diffusion

Table (2) also indicates that 100% conc. was the most active among all the examined concentrations since it could inhibit all of the ten *S.epidermidis* isolates by WD method. The less active concentration was 25% when it was examined by DD method and inhibited only half of the bacterial isolates.

TABLE 2: Numbers of *S.epidermidis* sensitive isolates against *C.longa* oil's concentrations by WD and DD methods.

Method	Oil's Concentrations			
	100%	50%	33.3%	25%
WD	10	9	8	6
DD	8	8	8	5

WD = Well Diffusion   DD = Disc Diffusion

In each of the two assays used, each concentration revealed a slightly higher activity as compared to the next one. The antibacterial activity of the investigated oil's concentrations was evaluated by determining the diameters means of inhibition zones (DMIZ) values against the two Gram positive *Staphylococcus* species. The activity of turmeric oil is highly detectable at the high concentration of the oil extract (concentrated oil 100%). When the comparison was made among the inhibitory act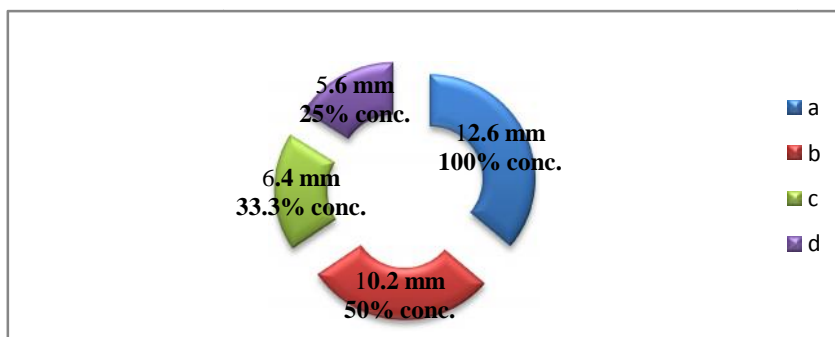ivity of the four oil's concentrations against *S.aureus* by using the two methods (WD, DD) (Table 3), it was found that there were high significant differences (P 0.01) between 1st and 3rd concentrations in the WD assay. Meanwhile, the 1st conc. itself exhibited the same significant differences with 4th conc. when both of the methods were used. The 2nd conc. showed significant differences (P 0.05) with 3rd conc. in the WD assay and with 4th conc. by using the two methods. On the other side, the significant difference was not demonstrable between the third and other concentrations by using DD method.

TABLE 3: Antibacterial activity of different concentrations of *C. longa* oil on *S. aureus* by WD and DD methods (diameters means of inhibition zones (mm))

Oil's Concentrations	Symbol	WD M±SD	Significance	DD M±SD	Significance
100%	A	12.6±1.5	c**d**	9.5±1.8	d**
50%	B	10.2±3.7	c* d*	7.7±3.1	d*
33.3%	C	6.4±5.6	a** b*	7.0±2.6	N.S.
25%	D	5.6±4.9	a** b*	4.9±3.4	a** b*

WD = Well Diffusion   DD = Disc Diffusion   M = Mean   SD = Standard Deviation
*   S. = Significant (P 0.05), **   H.S. = Highly Significant (P 0.01), N.S. = Not Significant (P 0.05)

By using the WD method, the concentrated oil (100%) showed the highest DMIZ value against *S.aureus* (12.6 mm) (Figure 1), but the lowest value was given by 25% conc. (4.9 mm) when DD method was used (Figure 2, 3).

Antibacterial potency of *Curcuma longa* L. oil extract against boils causing *Staphylococcus* species

FIGURE 1: Antibacterial activity of different concentrations of *C.longa* oil on *S.aureus* by using well diffusion method (a, b, c, d represent 1st, 2nd, 3rd,4th concentrations respectively) (DMIZ (mm)).
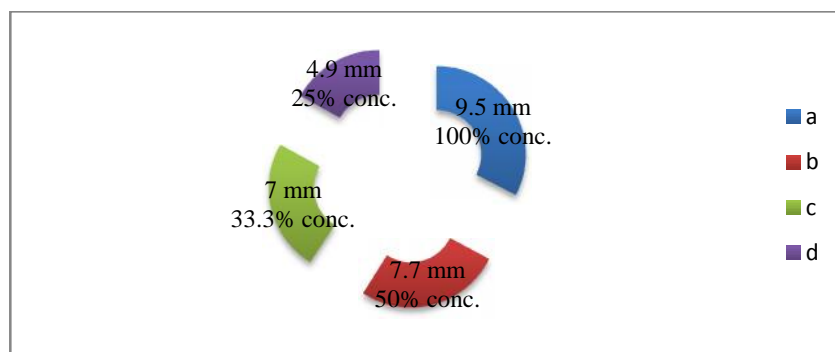
FIGURE 2: Antibacterial activity of different concentrations of *C.longa* oil on *S.aureus* by using disc diffusion method (a, b, c, d represent 1st, 2nd, 3rd,4th concentrations respectively) (DMIZ (mm)).
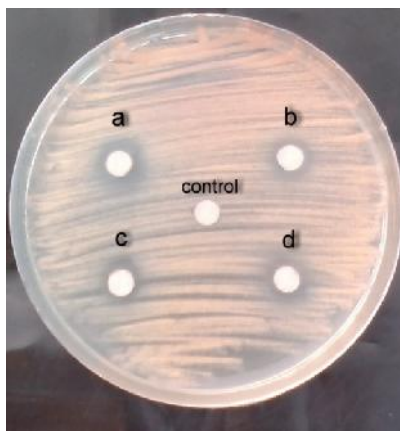
FIGURE 3: Anti- *S.aureus* activity of different concentrations of *C.longa* oil by disc diffusion method (a, b, c, d represent 1st, 2nd, 3rd,4th concentrations respectively).

TABLE 4: Antibacterial activity of different concentrations of *C. longa* oil on *S. epidermidis* by WD and DD methods (diameters means of inhibition zones (mm)

| Oil's Concentrations	Symbol	WD M±SD	Significances	DD M±SD	Significances
100%	a	16.5±4.1	c*d**	8.3±4.6	d**
50%	b	13.4±5.4	N.S.	7.3±4.1	d*
33.3%	c	11.0±6.3	a*	5.9±3.3	N.S.
25%	d	8.2±7.4	a**	3.6±3.8	b**a*

WD = Well Diffusion   DD = Disc Diffusion   M = Mean   SD = Standard Deviation *   S. = Significant (P 0.05),**   H.S. = Highly Significant (P 0.01), N.S. = Not Significant (P 0.05)

Table (4) showed the comparison between the oil's concentrations against *S.epidermidis*. The 1st conc. had significant differences (P 0.05) with 3rd conc. when the WD assay was used. On the other hand, the same concentration revealed highly significant differences (P 0.01) with 4th conc. by using the two methods. Both of the 2nd and 3rd concentrations did

not exhibit any significant differences with the three other concentrations by using WD, DD methods respectively. At last, 4th conc. showed significant differences (P 0.05) with 2nd conc. by using DD method. When noticing figur (4), it was found that

the maximum value of DMIZ was (16.5 mm) when the examined concentration was 100% by WD method, while 25% conc. had the minimum value (3.6 mm) when the DD method was used (Figure 5).
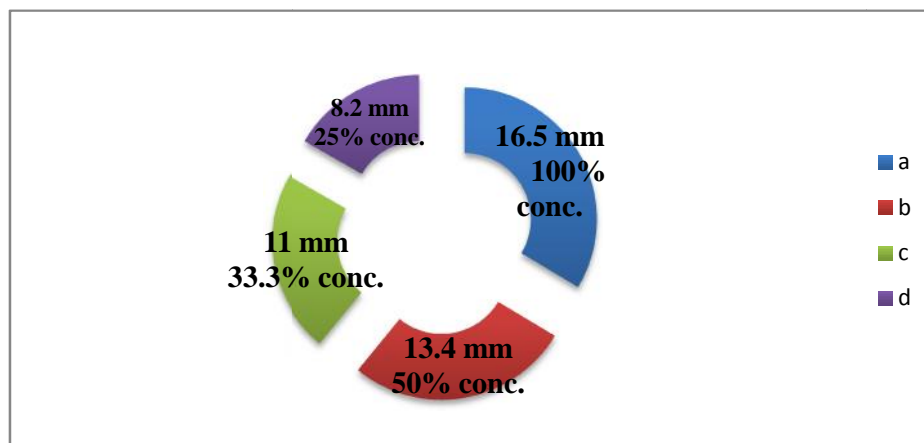
FIGURE 4: Antibacterial activity of different concentrations of *C.longa* oil on *S. epidermidis* by using well diffusion method (a, b, c, d represent 1st, 2nd, 3rd, 4th concentrations respectively) (DMIZ (mm))..
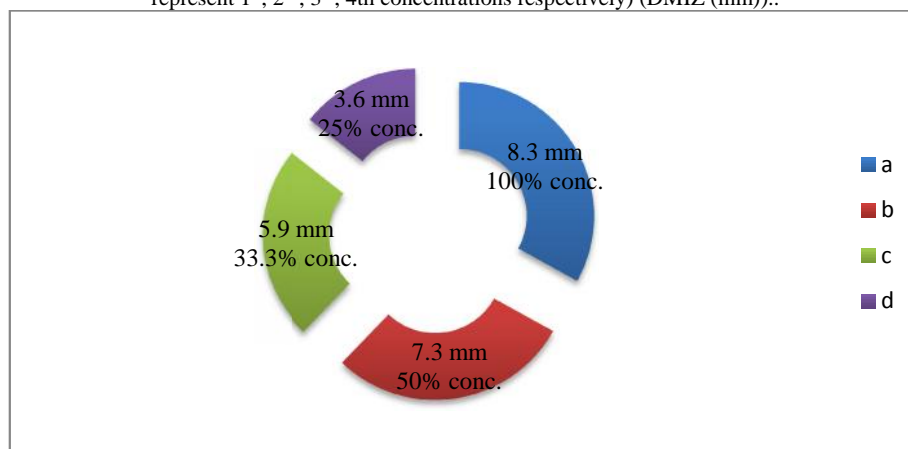
FIGURE 5: Antibacterial activity of different concentrations of *C.longa* oil on *S. epidermidis* by using disc diffusion method (a, b, c, d represent 1st, 2nd, 3rd,4th concentrations respectively) (DMIZ (mm)).

The comparison was also done between the efficiency of each of the two methods to evaluate the inhibitory activity of each of the examined oil's concentrations obtained against each of the two species that used in the study (Tables 5, 6).
Table (5) illustrated that there was a highly significant effect (P 0.01) of the method used for determining the antibacterial

activity of both the 1st (100%) and 2nd (50%) concentrations, while both of the 3rd (33.3%) and 4th (25%) concentrations exhibited inhibitory effects which were not significantly different (Figure 6).

TABLE 5: Comparison of the antibacterial activity of *C.longa* oil's concentrations on *S.aureus* between WD and DD methods.

Oil's Concentrations	Method	M±SD	P-value
100%	WD	12.6±1.5	0.001**
	DD	9.5±1.8	
50%	WD	10.2±3.7	0.001**
	DD	7.7±3.1	
33.3%	WD	6.4±5.6	N.S.
	DD	7.0±2.6	
25%	WD	5.6±4.9	N.S.
	DD	4.9±3.4	

WD = Well Diffusion   DD = Disc Diffusion   M = Mean   SD = Standard Deviation
**   H.S. = Highly Significant (P 0.01), N.S. = Not Significant (P 0.05)
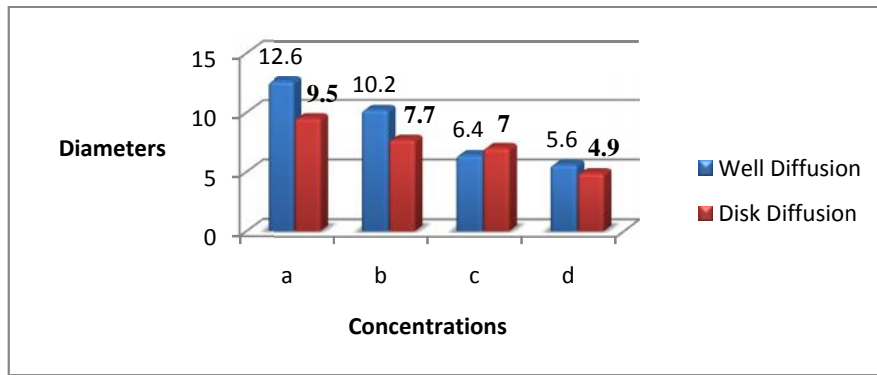
FIGURE 6: Comparison of the antibacterial activity of *C.longa* oil's concentrations on *S.aureus* between well diffusion and disc diffusion methods. (a, b, c, d represent 1st, 2nd, 3rd,4th concentrations respectively).

According to table (6) that showed the same comparison between the two methods against *S.epidermidis* isolates, there was high significant difference (P 0.01) between both methods for the 1st conc. A significant difference (P 0.05) was observed when comparing the two methods for the 3rd conc. However, changing the tested method had no significant influence in the antibacterial activity of both the 2nd and 4th concentrations (Figure 7, 8, 9).

TABLE 6: Comparison of the antibacterial activity of *C.longa* oil's concentrations on *S. epidermidis* between WD and DD methods

Oil's Concentrations	Method	M±SD	P-value
100%	WD	16.5±4.1	0.001**
	DD	8.3±4.6	
50%	WD	13.4±5.4	N.S.
	DD	7.3±4.1	
33.3%	WD	11.0±6.3	0.032*
	DD	5.9±3.3	
25%	WD	8.2±7.4	N.S.
	DD	3.6±3.8	

WD = Well Diffusion   DD = Disc Diffusion   M = Mean   SD = Standard Deviation
*   S. = Significant (P 0.05), **   H.S. = Highly Significant (P 0.01), N.S. = Not Significant (P 0.05)
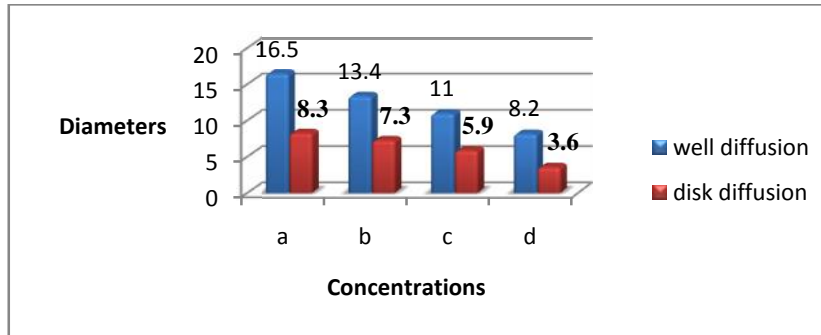
FIGURE 7: Comparison of the antibacterial activity of *C.longa* oil's concentrations on *S.epidermidis* between well diffusion and disc diffusion methods. (a, b, c, d represent 1st, 2nd, 3rd,4th concentrations respectively).
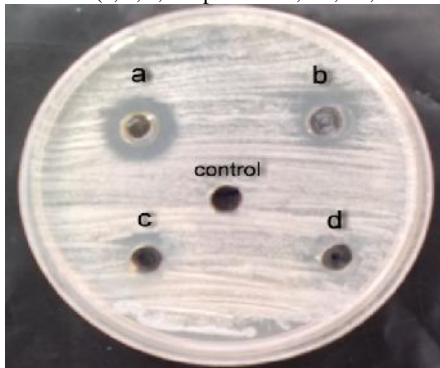
FIGURE 8: Anti- *S.epidermidis* activity of different concentrations of *C.longa* oil by well diffusion method (a, b, c, d represent 1st, 2nd, 3rd,4th concentrations respectively).
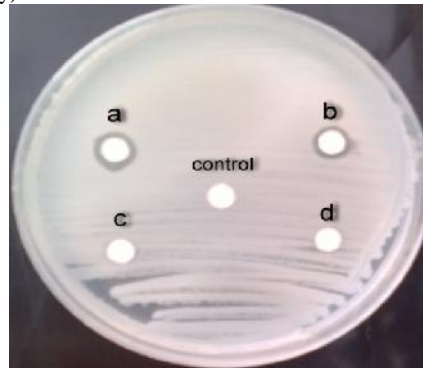
FIGURE 9: Anti- *S.epidermidis* activity of different concentrations of *C.longa* oil by disc diffusion method (a, b, c, d represent 1st, 2nd, 3rd,4th concentrations respectively).

The values of DMIZ estimated by the WD assay ranged from 5.6 to 12.6 mm for *S.aureus* and from 8.2 to 16.5 mm for *S.epidermidis*. While in the case of using DD assay, the DMIZ values ranged from 4.9 to 9.5 mm for *S.aureus* and from 3.6 to 8.3 for *S.epidermidis*. This presented that for both species, the inhibition zones values resulted from using WD method were higher than those obtained from DD method. As illustrated by the above results (tables 5, 6) (figures 6-9), all the concentrations of turmeric rhizome oil showed better inhibition when well diffusion assay was used. Consequently, we considered that this method was the most effective even at the lowest oil's concentrations.

DISCUSSION

Plant originated antimicrobial drugs are of interest because in part many human and animal pathogens show multi – drug resistant and in part certain antibiotics have undesirable side effects (Ahmad and Beg, 2001). Various health beneficial effects of turmeric are because it contains a number of secondary metabolites such as: monoterpenoids, sesquiterpenoids and curcuminoids which include: curcumin (also called diferuloylmethane), demethoxycurcumin, bisdemethoxycurcumin and tetrahydrocurcumin. These are yellowish pigments that have anti – inflammatory effects (Chainani-Wu, 2003; Park *et al*., 2005; Cikrikci *et al*., 2008). The antibacterial property of turmeric has also been attributed to the presence of an alkaloid and veleric acid, a byproduct from curcumin manufacture (Cikrikci *et al*., 2008; Pundir and Jain, 2010). Curcumin is the most important fraction which is responsible for a wide spectrum of biological activities of turmeric (Joe *et al*., 2004; Aggarwal *et al*., 2007; Cikrikci *et al*., 2008) such as antifungal, anti-inflammatory and antibacterial activities (Chattopadhyay *et al*., 2004; Siddiqui *et al*., 2006; Rai *et al*., 2008; Singh and Jain, 2011). Curcumin is considered to be a molecular constituent powerhouse, containing 20 different antibiotic molecules, it has a variety of potentially therapeutic properties, such as wound healing (Chainani-Wu, 2003; Cikrikci *et al*., 2008). Turmeric rhizomes contain two classes of secondary metabolites, curcuminoids and essential oils (Funk *et al*., 2010). The essential oil of *C. longa* rhizome has been studied in detail by a number of workers (Zwaving and Bos, 1992). The composition, structure as well as functional groups of the oils play an important role in determining the antibacterial activity, usually compounds with phenolic groups are most effective (Dorman and Deans, 2000; Rasooli, 2007). The bioassay guided fractionation procedure showed that the plant essential oil was rich in terpenes (monoterpenes, oxygenated monoterpenes and sesquiterpenes). At present, however, the mode of action of terpenic constituents on microorganisms is not fully understood (Norajit *et al*., 2007; Parveen *et al*., 2013). The major part of the rhizome oil contained sesquiterpenes (Leela *et al*., 2002; Chempakam and Parthasarathy, 2008; Shitou *et al*., 2011). Dried turmeric rhizomes usually yields 1.5 to 5% essential oils which are dominated by sesquiterpenes and are responsible for its aromatic taste and smell. Ar-turmerone, œ-turmerone and ß- turmerone are major ketonic sesquiterpenes of rhizome essential oils in Asia, and these compounds may account for at least 40% of essential oils of turmeric rhizomes (Golding *et al*., 1982; Sharma *et al*., 1997; Shitou et al., 2011). Some researches performed by Negi *et al*., (1999); Pundir and Jain, (2010); Lawhavinit *et al*., (2010) reported that the turmeric oils which include turmerone and curlone of turmeric possess excellent antibacterial action against a wide range of microbes such as: *Bacillus cereus, B.coagulans, B.subtilis, E.coli, P.aeruginosa* and *S.aureus.* Therefore, the results of the present study which revealed the anti-*Staphylococcus* effect of turmeric oil were similar to those demonstrated by the above researchers. Also, the antimicrobial activity of essential oil of turmeric with higher turmerone content was reported by Singh *et al*., (2005). This activity seemed to depend on total turmerone content in the plant oil extract. It is found that turmeric rhizome essential oil contain 49.76% of turmerone. Presence of this high amount of aromatic turmerone as the most abundant constituent in rhizome oil revealed that ar-turmerone is the major constituent of the rhizome oil of turmeric of different origins (Singh *et al*., 2005). The results obtained by our study was equivalent to a study done by Singh *et al*., (2002) which indicated that turmeric rhizome essential oil was more effective against Gram positive bacteria including *S.epidermidis* and pathogenic *S.aureus* which are actually responsible for the invasion and infection and have developed resistance to the standard antibiotics.

On the basis of a study which was done to evaluate the antibacterial potential of ten essential oils including turmeric rhizome oil against *Staphylococcus* sp. which was isolated from street foods, it was noticed that the bacteria represented high degrees of susceptibility (80%) towards these oils (Rath and Patra, 2012). The investigation of plant oil extract effective against *S.aureus* provides an example of prospecting for new compounds which may be particularly effective against infections that are currently difficult to treat (Sato *et al*., 1997). The essential oil of turmeric reported for anti-inflammatory, antibacterial and antifungal activities (Singh and Jain, 2011). Singh *et al*., (2011) have reported antimicrobial activity of the turmeric rhizome essential oil against *S.aureus* infections. Since *S.aureus* is often the main etiological agent associated with boil bacterial infections followed by *S.epidermidis*, it may explain the value of the plant in boils therapy, and these results are in agreement with our work. Our results support previous studies and revealed the potential use of turmeric essential oil as antibacterial agent against boil causing *Staphylococcus* sp. as well as these findings suggest an additional option to treat MRSA infections. Turmeric grown mainly in Thialand has been used in Thai herbal medicine for the treatment of various skin diseases (Lawhavinit *et al*., 2010). Curcumin may also applied topically to contract inflammation and irritation associated with inflammatory skin conditions and allergies (Mukhopadhyay *et al*., 1982). In the present study it was found that the undiluted oil (concentrated, 100%) had the best activity and the bigger inhibition zones compared to other concentrations (dilutions). In our knowledge, this is due to more antibacterial active contents of the concentrated oil which leads to the accumulation of these components. That means increasing the concentration may lead to elevating the amount of the active components which will increase the activity against bacteria. Highly detectable antibacterial action of the concentrated oil (100%) highlights the problem of deciding on the starting point of assaying oil extracts activity. There are many methods for determining antibacterial effectiveness. The NCCLS method for antibacterial susceptibility testing which is principally aimed at the testing of antibiotics has been modified for testing essential oils (EOs) (NCCLS, 2000; Rasooli, 2007). The most commonly used screens to determine antimicrobial susceptibility are disc or well diffusion assays, therefore, clinical microbiologists are very familiar with those assays (Tsuchiya *et al*., 1996). The well diffusion test in which the EO is deposited into wells cut into the agar can be used as a screening method when large numbers of Eos and / or large numbers of bacterial isolates are to be screened. It is worth noting that oil activity was estimated using well known and widely accepted method of inhibition zone measurement (Dorman and Deans, 2000; Rasooli, 2007). The well diffusion test is simple, easy to reproduce, inexpensive, easy both to read and interpret (Magaldi *et al*., 2004). These options were parallel with our findings. On the other hand, screening of EO for antibacterial activity is often done by the disc diffusion method which is generally used as a preliminary check for antibacterial activity prior to more detailed studies. Factors such as volume of EO placed on the paper discs, the thickness of the agar, layer and whether a solvent is used vary considerably between studies.

That means that this method is useful for selection between EOs but comparison of published data is not feasible. It is considered a quick measure of the inhibitory effects but it was not possible to demonstrate the antibacterial effect because the paper disc retains the active component and does not allow it to diffuse into the Muller-Hinton agar medium (Rasooli, 2007). Also, these causes were in agreement with our results.

The variations are of distinct importance in the study of biological and pharmacological activities of the essential oil, as the value of oil in aromatherapy has to be related to oil fraction and its chemical composition (Lawrence, 2000; Thongson *et al*., 2005). The differences in the antimicrobial activity of the oil might be due to the species of the microorganisms used and method of oil extraction (Gur *et al*., 2006). Other reasons such as time of plant collection, herbal material nature, plant part and climate (Parekh and Chanda, 2007), volume of inoculums, growth phase of microorganisms and culture medium used (Rios *et al*., 1988; Rasooli, 2007). One feature of test methods that varies considerably is whether or not an emulsifier or solvent is used to dissolve the EO or to stabilize it in water based culture media. Several substrates have been used for this purpose (Rasooli, 2007). In a study similar to ours, Pintore *et al*., (2002) used poly-ethylene glycol as an oil solvent. There are other factors which influence experimental outcomes during potency testing of natural antimicrobials. These include variability in composition or content of active agents that result from agronomic history variety differences and maturity of the plant material studied, physical and chemical characteristics, genus of microorganism, species and even strains susceptibility as well as previous culture history (Gill *et al*., 2002; Bagamboula *et al*., 2003; Rasooli, 2007). Thongson *et al*., (2005) pointed out that the antimicrobial efficacy results strictly dependent on concentration of the plant extract. The varying degrees of sensitivity of the bacterial test organisms may be due to both the intrinsic tolerance of microorganisms and the nature and combinations of phytocompounds present in the essential oil (Norajit *et al*., 2007; Parveen *et al*., 2013). The mechanism of antibacterial action of spices and derivatives is not yet clear (Lanciotti *et al*., 2004). Hypothesis have been proposed different workers (Odhav *et al*., 2002) which involve: hydrophobic 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 perturbation (Pundir and Jain, 2010). Some essential oils contain active components that influence certain metabolic functions of microbial cells. Wilkins and Board (1989); Parveen *et al*., (2013) suggested that antimicrobial activity of oils is may be due to impairment of variety of enzymes systems that are involved in the production of energy or synthesis of structural components in the microbial cells. Park *et al*., (2005) reported that Gram positive pathogenic bacteria display surface proteins that play important roles in the adhesion to specific organ tissues, the invasion of host cells, or the evasion of host-immune responses (Cossart and Jonquieres, 2000). These virulence-associated proteins are covalently anchored to bacterial cell wall peptidoglycan through a general sorting mechanism catalyzed by a superfamily of membrane-associated transpeptidases termed sortases (Schneewind *et al*., 1992). Two sortases isoforms, sortase A (SrtA) and sortase B (SrtB), have been identified in *S.aureus* (Mazmanian *et al*., 2002; Mazmanian *et al*., 2003).

Many pharmacological actions of turmeric have been reported to date. Park *et al*., (2005) concluded that curcumin isolated from *C.longa* rhizome is active against SrtA isoform which plays a critical role in the pathogenesis of Gram positive bacteria by modulating the ability of the bacterium to adhere to host tissue via the covalent anchoring of adhesions and other virulence-associated proteins to cell wall peptidoglycan (Mazmanian *et al*., 2003; Paterson and Mitchell, 2004).

CONCLUSIONS
Turmeric rhizome essential oil could dramatically inhibit boils causing *Staphylococcus* species in vitro. The inhibitory activity of the freshly extracted concentrated oil was higher than the diluted oil and this activity gradually decreased when the oil was diluted. The antibacterial potency of the oil was remarkable by using well diffusion method. Therefore, this assay was considered more effective than disc diffusion method.

RECOMMENDATIONS
Detailed studies should be carried out to determine accurately the bioactive compounds existed in the turmeric rhizome oil. Further researches on the mechanisms that explain the antibacterial activity shown by turmeric oil should be done. Promising use of turmeric rhizome essential oil as a novel natural effective antibacterial agent for the prevention and treatment of boils infections instead of the chemical drugs. Future clinical trials on the infected animals and humans (in vivo) are required to support the obtained outcomes. It is hoped that research institutes will continue their efforts to discover other new natural active compounds derived from aromatic plants with potent therapeutic action and less toxic than synthetic drugs.

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