Determination of antibacterial activity of *Curcuma longa* against selected food poisoning causing bacteria.

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Abstract: Plant derived medicine or herbs have made a huge contribution to human health. *Curcuma longa* was significantly proven to have antifungal and antiviral effects. In the present study, antibacterial activity *Curcuma longa* was investigated against selected bacteria by using disk diffusion method. Two types of solvent extraction were prepared, methanol and chloroform extraction. For the antibacterial screening, the extracts were tested against four selected bacteria; *Staphylococcus aereus, Listeria monocytogenes, Salmonella spp* and *Shigella spp* at 100% concentration. This is followed by Minimum Inhibitory Concentration (MIC) test at 25%, 50% and 100% to identify the lowest concentration which can inhibit the bacteria. The result showed that both extractions had antibacterial potential against both the gram negative bacteria and both gram positive bacteria. Thus, it is suggested that *Curcuma longa* leaves extracts has a potential to kill gram positive and gram negative pathogenic bacteria.

Keywords: antibacterial activity, *Curcuma longa,* disk diffusion method, MIC

Introduction

Over centuries ago, herbs and spices have been the most valuable treasure among countries. Plants have always been a rich source of biochemical compound. Many of these chemical compounds are useful drugs in themselves but safe to use because it is not harmful to human (Ody, 1993). Plant derived drugs remain an important resource, especially in developing countries, to tackle serious diseases.

The need for continuous research to discover new antimicrobial compounds with diverse chemical structure and mechanism of actions are very important (Ramzi *et al.*, 2005). It is because there always has been an increase in the incidence of new and re-emerging diseases and the major concern is the development of resistance to the antibiotics in current clinical use (Rojas *et al.* 2003).

Traditionally, plants have been a valuable source of natural products for maintaining human health with more intensive studies for natural therapies. Medicinal plants would be the best source to obtain a variety of drugs (Somchit *et al.*, 2002). About 80% of individuals from developed countries use traditional medicine, which has compounds derived from medicinal plants. Therefore such plants should be investigated to better understand their properties (Ellof, 1998).

The *Curcuma longa* plant which originated from India spread quickly to few parts of the world. The part that is commercially used is rhizome and the leaves (Kim, 2008). According to Oboh (1997), generally *Curcuma longa* are known to show high effects on bacteria especially on gram-positive organisms. *Curcuma longa* is one of the active antimicrobial agents. There are many usage of *Curcuma longa* such as anti-inflammatory, antioxidant, antiarthritic, topical antibacterial and antifungal, antifertility action and many more (Sharol Tignler., 1999). It is proven that the leaves extracts of these herbs is rich with bioactive compounds which have the capability to kill or inhibit the growth of pathogenic microorganism in human body (Fabricant and Farnsworth, 2005).

Curcuma longa has also been known to posses anti-inflammatory properties. Besides that *Curcuma longa* had also been used in pharmacology, pharmacokinetics, drug interactions and clinical trials (Grant and Schneider, 2000). Antimicrobial is a general

term that refers to a group of drugs that includes antibacterial, antibiotics, antifungal, antiprotozoals and antiviral. Antimicrobial drugs are derived from microorganisms that are used to fight infections caused by bacteria, fungi and viruses. Antimicrobial drugs designed to kill or inhibit the growth of microorganism.

Although numerous studies have been reported in the literature showing the antibacterial activity of *Curcuma longa*, however, there is no antibacterial activity was reported against food poisoning causing bacteria. The main objective of this study is to determine the antibacterial activity of *Curcuma longa leaves* extracts against food poisoning causing bacteria.

Materials and methods

Plants materials

The fresh green leaves of *Curcuma longa* were collected randomly in Sungai Buloh, Selangor Darul Ehsan. The leaves were put in the plastic container and stored in refrigerator for the experiment purposes.

Preparation of Plant Extracts

The fresh green leaves of *Curcuma longa* were washed under running tap water to remove contaminants, dust and debris on the surface of the leaves. Then the leaves were dried at the room temperature for three days. The dried leaves were blended into small particles and stored in an air tight bottle. 25g of dried samples were used for extraction. Two types of solvent were used in this study namely; methanol and chloroform.

Approximately 25g of the blended leaves were soaked in 500 ml of methanol with the ratio of 1:20 at room temperature. Then, the mixture was sealed tightly with aluminium foil and was left for 72 hours at room temperature. The mixture was mixed with sterile glass rod few times a day, to get a uniform mixing. After three days of soaking, the mixture was filtered through sterile cotton and filtered again with Whatman No.1 filter paper by using a vacuum pump filter aid to remove small particles. Later, the filtered methanol extractions were evaporated at 40° C under low pressure by using rotary evaporator to remove the excess methanol and to collect the crude extract. The same procedures of soaking, filtration and evaporation process were repeated for chloroform extraction.

Both crude extracts were dissolved in 5% of dimethyl sulfoxide (DMSO). Approximately, 0.90g of both crude extracts were weighed and dissolved in DMSO to a concentration of 1.0 g/ml for the subsequent antibacterial test. The crudes extracts of both methanol and chloroform were stored in sterile amber bottle and kept at -80°C for the further studies on antibacterial activity.

Culture preparation

Four bacteria were used in this study namely; *Staphylococcus aureus, Listeria monocytogenes, Salmonellaspp* and *Shigella spp*. All the bacteria were obtained from UNISEL Microbiology's laboratory. All the bacteria were subcultured into nutrient agar from the stock culture before it were cultured in Mueller Hinton broth. Then, the broth was left for overnight at 37°C. The bacterial suspension was compared to the 0.5 McFarland standards (Bauer *et al.*, 1966 and NurFizarini, 2007). This comparison was made when the tubes were viewed against a sheet of white paper on which sharp black lines was drawn. The turbidity standard was agitated on a vortex mixer immediately prior to use.

After the culture preparation, within 15 minutes after adjusting the turbidity of the inoculums suspension, a sterile cotton swab was dipped into the suspension. The cotton swab were pressed firmly against the inside wall of the tube and were rotatedto remove excess liquid. Then, the cotton swab was streaked over the entire surface of the Mueller Hinton Agar (MHA). The plates were left for few minutes to dry but not more than 15 minutes. This is to allow any surface moisture to be absorbed before applying the antibacterial impregnated discs (Chong *et al.*, 2008 and Nur Fizarini, 2007).

Disc Preparation

The extracts were prepared at 100% concentration. Sterile discs of 6mm diameter were loaded with the extracts and left dry under the laminar air flow. The discs were dispensed onto the Mueller Hinton agar surface by using sterile forceps. The discs were placed such that they have complete contact with the agar surface by touching the discs with forceps. Then, the plates were incubated at 37°C for 24

hours. During conducting this experiment, a triplicate was done for each microorganism to get a significant and accurate mean of inhibition zone (Chong *et al.*, 2008 and Nur Fizarini, 2007). Chloramphenicol and ampicilin antibiotic disk was used as positive control and DMSO as negative control. The screening of antibacterial activity was assessed based on the diameter of the clear zone surrounding the paper disc (including the disc diameter) in millimetre (mm). The results obtained were categorized into Sensitive (S), Intermediate (I) and Resistant (R) based on National Committee for Clinical Laboratory Standards, (2000).

Minimum Inhibitory Concentration (MIC)

The single concentration (100%) test was carried out before the Minimum Inhibitory Concentration (MIC) was determined by disc diffusion method. For MIC test, the most effective extract showing antibacterial activity in the 100% test were prepared into three different concentrations which is as follows; 25%, 50% and 100%. The experiment was carried out in triplicate. The sterile discs were soaked in these three different concentrations each and were left for dying under the laminar air. Then, the disc diffusion standard method was carried out according to the protocol standardized by National Committee Laboratory Standards (NCCLS). The results obtained were compared with each concentration to obtain the lowest concentration of the extract to inhibit the microbes used (Chong *et al.*, 2008 and Nor Fizarini, 2007).

Results and discussion

Antibacterial assay

In the present study, four food poisoning causing bacteria were tested for their sensitivity against two different extracts of *Curcuma longa* leaves. Table 1 shows the inhibitory effect of methanol and chloroform extracts of *Curcuma longa* leaves. Based on the result obtained, it showed that chloroform extract of *Curcuma longa* leaves has greater inhibitory effects towards the tested bacteria as compared to the methanol extract of *Curcuma longa* leaves. Methanol extract of *Curcuma longa* leaves inhibited both gram negative bacteria; *Salmonella spp.* and *Shigella spp.* and both gram positive bacteria; *Staphylococcus aureus* and *Listeria monocytogene*.

Table 1. Inhibition zone of methanol and chloroform extracts of Curcuma longa Leaves against selected bacteria

Microorganism	Zone of Inhibition (mm)				
			PC		NC
	Methanol Extract	Chloroform Extract	Ampicilin (10 µg)	Chloramphenic-ol (30 µg)	DMSO
Staphylococcus aureus	$9.00^{a} \pm 0.82$	$14.33^{b} \pm 0.56$	$22.00^{c} \pm 0.00$	-	-
Listeria monocytogene	$13.33^{a} \pm 0.56$	$14.33^{a} \pm 0.56$	-	$25.00^{b} \pm 0.00$	-
Salmonella spp.	$11.33^{a} \pm 0.55$	$13.33^{b} \pm 0.31$	-	$24.00^{c} \pm 0.00$	-
Shigella spp.	$15.00^{a} \pm 0.82$	$16.00^{b} \pm 0.00$	$30.00^{c} \pm 0.00$	-	-

* The zone of inhibition (mm) was expressed as the mean of the three replications ± the standard deviation. Abbreviations; a, b, c represents the statistical comparison made for the zone of inhibition for each microorganism. Mean with the different letters for each extracts and antibiotics are significantly different ($p < 0.05$).

Shigella spp exhibited the largest inhibition zone value of 15.00 mm, followed by *Listeria monocytogene* with 13.33 mm, *Salmonella spp* with 11.33 mm and *Staphylococcus aureus* with 9.00 mm.

On the other hand, chloroform extract of *Curcuma longa* leaves inhibited both the gram positive and gram negative bacteria. As for chloroform extract of *Curcuma longa* leaves, *Shigella spp* exhibited the largest inhibition zone value of 16.00 mm, followed by *Listeria monocytogene* and *Staphylococcus aureus* with each showing a value of 14.33 mm and lastly *Salmonella spp* with a value of 13.33 mm. It can be concluded that methanol extract and chloroform extract of *Curcuma longa* leaves were more efficient against both the gram negative bacteria. Besides that, based on the values exhibited, *Shigella* spp was the bacteria that have been efficiently inhibited by both the extract.

Based on the statistical analysis of one way ANOVA, there were significant differences ($p< 0.05$) between both methanol and chloroform extracts of *Curcuma longa* leaves against *Staphylococcus aureus*, *Salmonella spp*, *Shigella spp* and *Listeria monocytogene*. Further observation will also suggest that all the values of methanol and chloroform extracts of *Curcuma longa* showed significant differences ($p < 0.05$) against the respective antibiotics (Ampicilin and Chloramphenicol) used against each tested bacteria.

Minimum Inhibitory Concentration (MIC)

The effective form of extracts, which were methanol and chloroform extracts of *Curcuma longa* leaves, were brought to the next level of antibacterial assay test, Minimum Inhibitory Concentration (MIC). Table 2 shows the minimum inhibition concentration (MIC) of *Curcuma longa* leaves extracts against the four selected bacteria.

Based on the result, 25% of both extracts is the lowest concentration which able to inhibit all the four bacteria studied. All the four bacteria were susceptible or sensitive against methanol extract. The level of sensitivity of the microorganisms to the methanol extract of *Curcuma longa* leaves showed that *Shigella spp* is the most susceptible. Here, the extract exhibited a slightly larger value of inhibition zone relative to its inhibition zone value against other tested bacteria which was 9.33 mm in the lowest concentration of MIC, 25%. At the concentration of 50% and 100%, the extract shows an inhibition zone of 10.33 mm and 12.00 mm of diameter against *Shigella spp*. There is no significant difference ($p > 0.05$) seen between the inhibition zone value showed at 25% concentration for both methanol and chloroform extracts against *Shigella spp* where it exhibited a similar inhibition zone of 9.33 mm.

As for the chloroform extract of *Curcuma longa* leaves, the level of sensitivity of the microorganisms showed that *Shigella spp* is the most susceptible. In the concentration of 25%, 50% and 100%, the extract showed an inhibition zone value of 9.33 mm, 11.33 mm and 13.33 mm of diameter respectively against *Shigella spp*. As for 50% concentration, chloroform extract has the potential to act as an intermediate towards *Shigella spp*. However, at 100% concentration, chloroform extract inhibited effectively. Hence, it could be suggested that this extracts exhibited susceptible reaction towards this bacteria.

The result of this study showed effective antibacterial activity of both methanol and chloroform extracts of *Curcuma longa* leaves against selected bacteria that cause food poisoning. In this study, the leaves extracts were tested at 100% concentration (Table 1) initially and further tested with MIC test at three concentrations; 25%, 50% and 100% respectively (Table 2). It was found that methanol and chloroform extract of *Curcuma longa* leaves successfully inhibited all the test bacteria.

Being originated from the Zingeberaceae family, *Curcuma longa* leaves are known to contain a variety of chemical constituents; essential oils, including terpenes, alcohols, ketones, flavonoids, carotenoids, phytoestrogens and others. Some of the major components present in the essential oil of the *Curcuma longa* leaves are several monoterpenes and sesquiterpens compounds such as zingiberene; ar-, a- and ß-turmerone. The other major substance that is present in *Curcuma longa* leaves is curcuminoids. All the compounds of essential oil play a major role in the antimicrobial activity. For example, d-limonene from the essential oil inhibited five strains of *P. acnes* which contributed to the anti inflammatory and antimicrobial activity (Habsah *et al*., 2000).

The essential oil which possesses an antimicrobial antimicrobial activity contains both polar constituent; terpenes and non polar constituent; ß- turmerone . Terpenes, an aldehyde in nature is slightly soluble in water and ether and soluble in organic solvents. ß- turmerone on the other hand is a hydrocarbon which is insoluble in

water (Habsah *et al*., 2006). Methanol is a polar solvent which will extract polar compounds, while

Table 2. Minimum inhibition zone of different concentration of *Curcuma longa* leaves extracts

Microorganism	Zone of Inhibition (mm)								
	Different Concentration of Extracts						PC		NC
	Methanol Extract			Chloroform Extract			Amp	Chlo	DMSO
	25%	50%	100%	25%	50%	100%			
Staphylococcus aureus	5.67[a] ± 0.43	7.00[b] ± 0.00	9.00[d] ± 0.82	7.67[c] ± 0.41	9.67[e] ± 0.40	11.00[f] ± 0.82	28.00[g] ± 0.00	-	-
Listeria monocytogene	7.00[a] ± 0.00	8.67[c] ± 0.41	10.00[e] ± 0.82	8.00[b] ± 0.82	9.33[d] ± 0.53	11.33[f] ± 0.55	-	24.00[g] ± 0.00	-
Salmonella spp	9.00[a] ± 0.00	10.00[b] ± 0.00	10.00[b] ± 0.00	9.00[a] ± 0.00	10.67[c] ± 0.56	11.00[d] ± 0.56	-	25.00[e] ± 0.00	-
Shigella spp	9.33[a] ± 0.00	10.33[b] ± 0.00	12.00[d] ± 0.39	9.33[a] ± 0.53	11.33[c] ± 0.00	13.33[e] ± 0.82	28.00[f] ± 0.00	-	-

* The zone of inhibition (mm) was expressed as the mean of the three replications ± the standard deviation. Abbreviations; a, b, c, d, e, f represents the statistical comparison made for the zone of inhibition for each microorganism. Mean with the different letters for each extracts and antibiotics are significantly different ($p < 0.05$).

chloroform which exhibit both polar and non polar properties will extract both polar and non polar compounds.

Therefore, a larger amount of the essential oil will be extracted by the chloroform as compared to methanol. This explains the reason for chloroform extract of *Curcuma longa* leaves being more efficient; ability to inhibit all the bacteria employed as compared to the methanol extract of *Curcuma longa* leaves.

Gram negative bacteria contain an outer phospholipidic membrane which carries the structural lipopolysaccharide components. These results in a cell wall that is impermeable to lipophilic solutes; inability of a chemical compound to dissolve in fats, oils, lipids and non-polar solvents (Joanne *et al.*, 2008). Methanol extracts which are composed of polar compounds were able to permeate through the membrane. Hence, this explains the ability of methanol extracts to exhibit a better inhibition zone value in gram negative bacteria as compared chloroform extracts.

Meanwhile, gram positive bacteria contain an outer peptidoglycan layer which is a permeable barrier (Joanne *et al.*, 2008). Since it is a permeable membrane, thus it is able to allow both the methanol and chloroform extract permeate through the membrane. As discussed before, chloroform will tend to extract more of the essential oil ascompared to methanol. Thus, it's produce a better inhibition in gram positive bacteria. This relation explains the reason for the ability of chloroform extracts to exhibit a better inhibition zone value in gram positive bacteria as compared methanol extracts.

Conclusion

Based on the result of this study, it can be concluded that *Curcuma longa* leaves has great potential to act as antimicrobial agent against microorganisms. From the observation, both methanol and chloroform extracts of *Curcuma longa* leaves exhibited antimicrobial activity against four selected bacteria with susceptible, intermediate and resistant action as compared to the standard antibiotic used (ampicillin and chloramphenicol) in this study.

The disc diffusion result shows,methanol and chloroform extract of *Curcuma longa* leaves inhibited all four microorganisms namely *Staphylococcus aureus*, *Listeria monocytogene*, *Salmonella spp* and *Shigella spp* at 100% concentration. However, chloroform extract of *Curcuma longa* leaves were seen to be much effective against gram negative bacteria as compared to gram positive bacteria. Both the

extracts however showed a minimum inhibitory concentration (MIC) of 25% against all bacteria tested for MIC. This result supports the fact that *Curcuma longa* leaves have the ability to inhibit pathogenic microorganisms that are prone to cause food poisoning.

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