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IN VITRO ANTIMICROBIAL ACTIVITY OF FERMENTED SPICES AND *CAPSICUM FRUTESCENS*AGAINST MULTI DRUG RESISTANCE CLINICAL ISOLATE AND STANDARD REFERENCE BACTERIA

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ABSTRACT

Introduction: Food preservation is required to maintain for a long period of time. Traditional organic food preservative, "Datta" is spice mainly made up of Chili Peppers which frequently used in southern and western part of Ethiopia. Datta can be consumed almost with every kind of foods and it is believed as appetizer and antimicrobial agent against food borne pathogen. This study aimed to assess *in vitro* antimicrobial activity of fermented condiment and Capsicum *frutescens*against multi drug resistance clinical isolate and standard reference bacteria.

Method: Datta samples collected from different level hotels and *Capsicum frutescens* (Chili peppers) were extractedin different solvents.Agar well diffusion assay was used to determine antimicrobial activity and minimum inhibitory concentration (MIC) and minimum bactericidal concentration was determined by tube dilution method. One way analysis of variant was used in comparison of the finding.

Results: Extracted fermented condiment (Datta) sample and Chili Pepper showed antimicrobial activities against multidrug resistant clinical isolate and standard reference bacteria in well diffusion assay. Datta extract showed MIC ranged from 25 mg/L to 66.7 mg/L and MBC ranged from 25 mg/L to 100 mg/L. The Datta and Chili pepper extracts showed high antimicrobial activities against standard *Staphylococcus aureus*. The water based extract of Datta sample were exhibited significantly low antimicrobial activities (P=0.000) as compared to the other extraction solvents.

Conclusion: Water was weak extractor of active compounds having antimicrobial activities. Reference *S. aureus*wasmore susceptible organism while ATCC *Salmonella enteritidis*and clinical isolated multi-drug resistant *E. coli* less susceptible. The traditional use of fermented condiment for food preservation by the local people is supported by this study.

Key words: Antimicrobial activity; Chili Pepper extract; Fermented condiment; Minimum bactericidal concentration; Minimum inhibition contraction

ACTIVITÉ ANTIMICROBIENNE EN VITRO D'ÉPICES FERMENTÉES ET DE FRUITS DE CAPSICUME POUR LA RÉSISTANCE AUX MÉDICAMENTS ISOLATE CLINIQUE ET BACTÉRIES DE RÉFÉRENCE STANDARD

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ABSTRAIT

Introduction: La conservation des aliments est nécessaire pour maintenir pendant une longue période de temps. Conservateur de nourriture organiqu et raditionnel, "Datta" est l'épicé compose principalement de Chili Peppers qui fréquemment utilize dans le sud et l'ouest de l'Ethiopie. Datta peu têtre consommé presque avec toutes sortes d'aliments et on le croit comme un apéritif et un agent antimicrobien contre l'agent pathogène alimentaire. Cette etude visait à évaluer l'activité antimicrobienne *in vitro* du condiment fermenté et *Capsicum frutescens* contre l'isolement clinique de résistance aux médicaments multiples et les bactéries de référence standard.

Méthode: Les échantillons de Datta prélevés dans des hôtels de différents niveaux et *Capsicum frutescens* (Chili Peppers) ont été extraits dans différents solvants. Un dosage de diffusion de puits a été utilisé pour determiner l'activité antimicrobienne et la concentration inhibitrice minimale (MIC) et la concentration bactericide minimale a été determine par la méthode de dilution du tube. Une analyse à sens unique de la variante a été utilisée en comparaison de la découverte.

Résultats: L'échantillon extrait de condiments fermentés (Datta) et Chili Pepper ont montré des activités antimicrobiennes contre l'isolement Clinique résistant aux médicaments multiples et les bactéries de référence standard dans le dosage par diffusion de puits. L'extrait de Datta a montréque le MIC variait de 25 mg / L à 66,7 mg / L et le MBC variait de 25 mg / L à 100 mg / L. Les extraits de poisson de Datta et de Chili ont montré des activités antimicrobiennes élevées contre *Staphylococcus aureus* standard. L'extrait à base d'eau de l'échantillon de Datta a montré des activités antimicrobiennes significativement faibles (P = 0,000) par rapport aux autres solvants d'extraction.

Conclusion: L'eauétaitun extracteur faible de composes actifs ayant des activités antimicrobiennes. Référence *S. aureus* était un organisme plus susceptible tandisque ATCC *Salmonella enteritidis* et *E.coli. E. coli* résistant aux médicaments multiples isolés était moins susceptible. L'utilisation traditionnelle du condiment fermenté pour la conservation des aliments par les populations locales est soutenue par cette étude.

Mots clés: Activitéantimicrobienne; Extrait de poivre de piment; Condiment fermenté; Concentration bactéricide minimum; Contraction minimaled'inhibition

INTRODUCTION

Since ancient time, peoples have been using spices to prevent off-odor, off-flavors and spoilage of foods [1]. Besides flavoring food, currently spices have become an integral part of daily requirements such as for food preservation, cosmetics, medicinal preparation, bakery goods, perfumery, and various other products [2]. The preparation of this spices vary from place to place. One of well now and the most common spice that is used in most type of food in Ethiopia is known as “Datta” in southern part of the country and “Kotchkocha” in western part in Afan Oromo language. Datta is not heat processed and the main ingredient of this spice is pasted Chili Pepper whereas other ingredient such as Garlic, Ginger, Coriander, and Basil are added in limited amount. All these ingredients crushed together by simple grinding stone until it become semisolid. The Datta acquires original precursor chili pepper color, it became green if it is made of fresh green chili pepper or become red in color if red chili peppers are used [3, 4].

Some foods are not easily contaminated with microorganisms. This may be due to food intrinsic antimicrobial agent of raw materials that have inhibitory effect on growth of microorganisms. Many secondary metabolites of plants are antibiotic, protecting the plants against different microorganisms [5, 6]. Therefore, herbs are now more focused than ever because they have the capability of producing important metabolites which used as medicine or as precursor for many pharmacological products. Without specific knowledge of their cellular action or mechanisms phytochemicals have been considered possible drugs for times.

Chili peppers are also used worldwide in foods for their pungent flavor, aroma, and to prolong food spoilage. It is one of spice which considered as preservative as well as appetizer by the users. Every country in the world has its own way in making different types of foods and spice. Ethiopia is a country with different ethnic groups with different culture, preparation of spices varies in different part of the country. This study is conducted on Datta which is the most dominant and widely used spice product of pepper known in southern and western part of Ethiopia [3, 4].

Different study showed as Chili Pepper which is the major precursor of Datta has antimicrobial activities on different microorganisms [7, 8, 9]. The Capsaicin present in Chili Pepper is has

antimicrobial activities in addition to other secondary metabolites. Little is known about antimicrobial activities of Datta and scientific evidence is required to confirm its food preservative characteristic of the spice. Searching of effective and safe food preservatives has non debatable benefits now day. There are new concerns about food safety and preservatives due to increasing occurrences of new food-borne disease outbreaks caused by drug resistant pathogenic microorganisms. This advances considerable challenges, mainly since there is increasing unease regarding the use of chemical preservatives and artificial antimicrobials to inhibit growth of spoilage microorganisms [10]. These chemical preservatives may have so many side effects as compared to organic preservatives that has a little effect on food flavors are always needed. Spices Chili Peppers may be used as organic food preservative because people are using this condiment during eating delayed food. Currently, there is growing interest in using natural preservatives compounds as alternatives to synthetic compounds for food preservation [11]. In this study, the antimicrobial activity of Chili Pepper spices is going to be investigated as an alternative to antibiotics and food preservative option. The aim of this study is to assess *in vitro* antimicrobial activity of fermented condiment and chili pepper against multi drug resistance clinical isolate and standard reference bacteria.

MATERIALS AND METHODS

Study design, setting and period

Experimental study deign was carried out to asses antimicrobial activity Chili Pepper extract and Arba Minch town Datta collected from high, medium and low level Hotels. The study was conducted in Arba Minch town. The source of Datta's Chili Peppers for Arba Minch town is agriculture land of highland areas surrounding the town and the source of Datta samples were Hotels in the town with different level. Arba Minch town is located 500 kms south of Addis Ababa in southern nation nationality region of Ethiopia situated in the great African rift valley in elevation of 1285 meters above sea level with average temperature of about 29.7^{o}C and the average annual rain fall of 900mm.

Sample collection

To obtain representative sample, Datta samples for assessing antimicrobial activities against ATCC and MDR clinical bacteria isolates were collected from hotels having different level. The Datta samples were collected using sterile and leak proof containers and transported to the microbiology laboratory using icebox, and kept in refrigerator during analysis periods. During collection, semi-structured questionnaire and observation check list were used to characterize the Datta samples.

Fresh chili peppers (*Capsicum frutescens*) which used as ingredient for Datta preparation were collected and characterized by local experts. Then the collected peppers were transported to laboratory in aseptic manner. The species name of collected the chili pepper is determined by the help of Taxonomist. Finally collected sample were extracted according to method discussed below.

Experimental organisms

The screening for antibacterial activities of Datta and Chili pepper crude extracts were carried out by using three bacterial pure cultures: *Staphylococcus aureus* (ATCC® 25923™), *Salmonella enterica subsp. enterica*(ATCC® 13311™) and *Klebsiellapneumoniae* (ATCC® 700603™) and three multi drug resistance clinical bacteria isolates particularly Methicillin resistant *Staphylococcus aureus*(MRSA), *Pseudomonas aeruginosa,* and *Escherichia coli*. The organisms were regularly sub-cultures on Nutrient agar slant until screening for antimicrobial activity.

Datta and Chili Pepper extraction

The collected Datta sample in each hotel was separated into two sterile containers. The first container was used for microbiological characterization of the sample while the second container was for antimicrobial activities. For antimicrobial analysis, 10% w/v proportion was used to extract in four different extraction solvents (Distilled water, Acetone, Ethanol, and Methanol). Then dissolved solution of each sample was kept in orbital shaker for 24 hrs at room temperature. Finally the solution was filtered by Whatman No. 1 filter paper and stored in deep freeze at negative 20^{o}C for further use of antimicrobial analysis [6].

Chili Pepper extraction was performed in six different solvents: Distilled water, Acetone, Ethanol, Methanol, Ethyl acetate, and Chloroform. The collected fresh chili pepper was washed twice in running water and once in distilled. Then it was crushed in disinfected

Morton and pestle. Then 7.5gm of crushed chili pepper will be dissolved in 75ml each solvents to obtain 10% w/v. Then the dissolved solutions were kept on orbital shaker for 24hr. Then the extract was filtered with sterile Whatman No. 1 filter paper and stored in deep freeze at -20^{o}C for further use of antimicrobial analysis.

Agar well diffusion assay of Datta sample and chili pepper extracts

Datta sample and Chili pepper crude extract was obtained aforementioned methods was evaluated for antimicrobial activity by agar well diffusion assay. Muller Hilton agar (Oxoid Limited, CM0337) is used to perform antimicrobial activity of the extracts. Then agar well diffusion assay was carried out according to method described in clinical and laboratory standards institute [12]. Diffusion well of approximately 6mm diameter was prepared by a sterile micro pipette tip. A 0.5 McFarland standards diluted suspension of each test microorganisms was evenly inoculated on Muller Hilton agar. Then 50µl of the extracts from each sample of Datta and Chili Pepper extract was carefully filled in the well. Additional wells were prepared and filled with each solvent used as negative control. Then the inoculated agar plates were incubated at 37^{o}C for 24 hours. All the tests were conducted in triplicate and the average of the three measurements was used to present the results.

Determination of minimum inhibitory concentration of Datta sample and chili pepper extracts

Minimum inhibitory concentration (MIC) of Datta sample and chili pepper extract was determined by tube dilution method. The extracted Datta and Chili Pepper were diluted from 100mg/L to 12.5mg/L in nutrient broth (Oxoid). To each test tube with extract, a loop full of 10^{6} bacteria suspension per ml fresh nutrient broth inoculated in each tube. Then the culture tubes will be incubated at 37^{o}C for 24 hours. After the period of incubation the tubes are checked for turbidity that indicates the growth of bacteria. Then the lowest concentration of Datta sample and pepper extract inhibits growth of the test organisms was considered as the MIC for the respective organisms. To confirm the inhibition, then a loop full of the incubated inoculum was sub-cultured on nutrient agar [5].

Determination of minimum bactericidal concentration of Datta sample and chili pepper extracts

Minimum bactericidal concentration (MBC) of Datta samples and Chili Pepper extract was determined by tube dilution method followed by confirmation on agar plates. The MIC and the two consecutive preceding concentrations were used to determine MBC. The three concentrations of Datta and Chili Pepper extract were adjusted in Nutrient broth and inoculated with loop full of test organisms. Then the inoculam was incubated at 37^{o}C for 24 hours. After the period of incubation the loop full of the well mixed inoculam from nutrient broth was transferred to nutrient agar and incubated at 37^{o}C for 24 hours. Then the plates were examined for visible colony and the MBC is the lowest concentration that demonstrates no growth of colony.

Statistical Analysis

Collected data was entered to excel and exported to SPSS version 20.0 for further analysis. Descriptive statistics such as means and standard deviations were calculated. One way ANOVA followed by Tukey's test was used to compare extracts and the difference in the susceptibility of the test microorganisms. The 95% level of confidence (P-value ≤ 0.05) was considered as statistically significant.

RESULTS

Characteristic of collected Datta sample

A total of nine Datta samples were collected in different level hotels of Arba Minch town. Among collected Datta samples, three of them have red color; the other three of them have green color while the others have gray and brown color. According to information collected from the Datta owners of each hotels, the Datta sample were prepared mainly from Chile pepper while some other spices such as garlic, ginger, coriander, and other locally available plant spices were added. The average storage time after preparation of the Datta samples were 2 weeks with a range of 3 days to the 30 days. About one third of the Datta samples collected from low profile hotel were stored at room temperature while the rests were stored in Refrigerator.

Antimicrobial activity of Datta extract by well diffusion assay

The collected Datta samples were subjected to four solvent extractions particularly with Acetone, Ethanol, Methanol and distilled water. Stock concentration of 100mg/L Datta extract was used for antimicrobial analysis by agar well diffusion assay against two set of bacteria. The test organisms were both multi drug resistant clinical isolates and standard (ATCC) bacteria. There is no significance difference in antimicrobial activities among the three profile hotels (P=0.93).

TABLE 1: ZONE OF INHIBITION OF DATTA SAMPLE AGAINST ATCC AND MULTI-DRUG RESISTANT CLINICAL BACTERIAL ISOLATES (100 MG/L).

Datta Source	Extract solvent	Zone of inhibition (mm) (Mean ± SD).					
		ATCC Bacteria			MDR Clinical isolates Bacteria		
		S. aureus	*K. pneumoniae*	*S. enteritidis*	*P. aeruginosa*	*E .coli*	*S. aureus*
HLH	Water	14.3±3.5	-	-	-	-	-
	Acetone	23.7±1.5	12.7±0.8	13*	18.3±3.6	9±5.8	19.3±1.8
	Ethanol	22±3	15.2±1	7±4.3	15.7±1.5	8.3±5.1	16.7±1
	Methanol	20.7±2.3	15.3±1.1	11.3±1	13±1.8	8±4.9	16.3±2.3
MLH	Water	-	-	-	-	12*	-
	Acetone	18.7±2.2	14.3±2	13.5**	19.3±2.7	11*	15±2.1
	Ethanol	21.3±3.9	16.3±1.6	12*	18.7±3.5	10±6.1	14±0
	Methanol	20.7±3	14.3±1	15.7±04	18.3±2.2	11.7±0.8	15±1.1
LLH	Water	9.3±2.4	-	-	-	-	11*
	Acetone	26.3±0.4	14.3±0.8	12±1.2	16.3±3.6	12.5**	14.3±0.8
	Ethanol	21.3±1.4	12±0.7	12.0**	16.7±0.8	8±4.9	16.7±1
	Methanol	21.3±2.4	12.7±1	12.7±1	15±1.4	11*	16.7±4.2

HLH= high level hotel MLH=medium level hotel LLH=low level hotel

***antimicrobial activity was observed only in one sample, **antimicrobial activity was observed only in two samples**

The water based extract of Datta sample were exhibited significantly low antimicrobial activities (P=0.000) as compared to the other extraction solvents. The highest (14.3±3.5) zone of inhibition of water based extract was observed in high level hotel (HLH) Datta sample followed by medium profile hotel (MLH) against ATCC® 25923™ *S. aureus.* Only one sample of the medium, and low profile hotels showed antimicrobial activity against clinical isolates *E. coli* and*S. aureus* respectively (**Table 1**). The alcohol based (ethanol and methanol) Datta extract showed no difference in antimicrobial activities (P=0.49) in agar well diffusion assay. The highest 26.3±0.3 zone of inhibition of Acetone based extract was observed in LLH against ATCC® 25923™ *S. aureus* whereas the minimum was exhibited against *Salmonella enteritidis* (ATCC-1331). Methanol based extract showed overall average of 21, 16, and 15.4mm zone of inhibition against ATCC-25923 *S. aureus,* Methicillin-resistant *S. aureus* (MRSA), and clinical isolate *Pseudomonas* specious respectively. Acetone based extract exhibited 23, 18, and 16.2mm average inhibition zone against ATCC *S. aureus,* clinical isolate *Pseudomonas* spp., and MRSA respectively (**Table 1**). Pair wise comparison of ANOVA this study showed that the test organisms' susceptibility against Datta samples extract varies. As a whole ATCC *S. aureus*was more susceptible than the other test organisms (P<0.05). Similarly there is significant difference between ATCC *Salmonella enteritidis* and clinical isolate *Pseudomonas* spp. (p=0.028), and ATCC *Salmonella enteritidis* and MRSA (P=0.037). On the other hand clinical isolate *Pseudomonas* spp. showed significant difference with ATCC *S. enteritidis*and clinical isolate *E.* coli whereas *E. coli* susceptibility varies with MRSA as well (P=0.036).

Antimicrobial activity of Chili Pepper extract by well diffusion assay

Similar to Datta samples, fresh chili pepper (*Capsicum frutescens*) family name of Solanaceaewas extracted in six different extraction solvents (acetone, ethyl acetate, chloroform, ethanol, methanol and distilled water) and evaluated for antimicrobial activity by agar well diffusion assay. No zone of inhibition was observed for water based chili extract against

all test organisms at 100mg/L. All other extracts showed different zone of inhibition on test bacteria. The largest average zone of inhibition (28mm) was observed by acetone based extract against ATCC *S. aureus* while the smallest (11mm) zone of inhibition was obtained in methanol based extract against clinical isolate MDR *P. aeruginosa*. Like in the case of Datta extract overall result showed that ATCC *S. aureus* was the most susceptible followed by clinical isolate MDR *P. aeruginosa* whereas ATCC *S. enteritidis* and MRSA were the most resistant bacteria for chili extracts (**Table 2**).

TABLE 2: ZONE OF INHIBITION OF CHILLI EXTRACTS AGAINST ATCC AND MULTI-DRUG RESISTANT CLINICAL BACTERIAL ISOLATES (100 MG/L)

Extract solvent	Zone of inhibition (mm)					
	ATCC Bacteria			MDR Clinical isolates Bacteria		
	S. aureus	*K. pneumonia*	*S. enteritidis*	*P. aeruginosa*	*E .coli*	*S. aureus*
Water	-	-	-	-	-	-
Acetone	**28**	**19**	**15**	**21**	**17**	**17**
Ethanol	**27**	**18**	**12**	**19**	**18**	**12**
Methanol	**24**	**12**	**13**	**11**	**16**	**13**
Ethyl Acetate	**26**	**12**	**12**	**15**	**17**	**15**
Chloroform	**23**	**13**	**17**	**22**	**13**	**12**

Antimicrobial activities of chili extract differ based on extraction solvent. Chili Pepper water based extract showed week antimicrobial activity than the other solvents (p=0.000) similar to Datta extract. ANOVA pair wise comparison also indicated significance difference between acetone and methanol. Acetone is better extraction solvent than distilled water and methanol while there was no significant difference with other solvents. As a whole there was no significant difference (P=0.12) in susceptibility of test bacteria for chili extracts.

Minimum inhibitory and minimum bactericidal concentration of Datta extract

Minimum inhibitory concentration (MIC) and minimum bactericidal concentrations (MBC) for ethanol and acetone based extract were performed by four serially diluted of Datta extracts. The result of MIC of Datta extract in both extractions solvent ranges from 25 mg/L to 66.7 mg/L. Similarly, the MBC of the extracts indicates the range from 25mg/L to 100m/L. The overview of the finding showed relatively minimum concentration was required to inhibit or kill ATCC *S. aureus* than the other test bacteria.

TABLE 3: MINIMUM INHIBITORY AND MINIMUM BACTERICIDAL CONCENTRATION OF DATTA SAMPLE

Datta Source	MIC/ MBC	Extract solvent	MIC and MBC (mg/L)					
			ATCC Bacteria			MDR Clinical isolates Bacteria		
			S. aureus	*K. pneumonia*	*S. enteritidis*	*P. aeruginosa*	*E .coli*	*S. aureus*
HLH	*MIC*	**Ethanol**	**25±0**	**33.3±10.2**	**66.7±20.4**	**41.7±10.2**	**41.7±10.2**	**58.3±16.6**
		Acetone	**33.3±10.2**	**41.7±10.2**	**25±0**	**25±0**	**25±0**	**33.3±10.2**
	MBC	**Ethanol**	**33.3±10.2**	**33.3±10.2**	**83.3±20.4**	**33.3±10.2**	**50±0**	**66.7±20.4**
		Acetone	**58.3±27**	**75±10.3**	**25±0**	**41.7±10.2**	**41.7±10.2**	**50±0**
MLH	*MIC*	**Ethanol**	**33.3±10.2**	**33.3±10.2**	**50±0**	**50±0**	**41.7±10.2**	**25±0**
		Acetone	**41.7±10.2**	**33.3±10.2**	**41.7±10.2**	**33.3±10.2**	**25±0**	**50±0**
	MBC	**Ethanol**	**58.3±27**	**33.3±10.2**	**100±0**	**66.7±20.4**	**58.3±27**	**33.3±10.2**
		Acetone	**50±0**	**58.3±27**	**75±103.8**	**58.3±27**	**33.3±10.2**	**83.3±10.2**
LLH	*MIC*	**Ethanol**	**50±0**	**41.7±10.2**	**25±0**	**58.3±27**	**25±0**	**66.7±20.4**
		Acetone	**33.3±10.2**	**33.3±10.2**	**33.3±10.2**	**25±0**	**25±0**	**25±0**
	MBC	**Ethanol**	**83.3±20.4**	**58.3±27**	**66.7±20.4**	**66.7±20.4**	**33.3±10.2**	**83.3±20.4**
		Acetone	**50±0**	**58.3±27**	**50±0**	**33.3±10.2**	**33.3±10.2**	**25±0**

There was no major difference between the three profile hotels. The result shows MIC of ethanol extract range from 25mg/L to 66.7mg/L for tasted organisms and acetone extract had MIC that ranges from 25mg/L to 50mg/L test bacteria specious. The overall MBC for ethanol extract has value range from 33.3 mg/L to 100mg/L whereas acetone extract had MBC that range from 25mg/L to 83.3 mg/L (**Table 3**).

Minimum inhibitory and minimum bactericidal concentration of Chili pepper extract

For Chili Pepper extracts the minimum inhibitory and minimum bactericidal concentration was determined for five solvents. In general, ATCC *S. aureus* was inhibited by minimum concentration as compared to the other test organisms. Similar to MIC, the least concentration was required to kill ATCC *S. aureus* as compared to other test bacteria specious. The overview of the result showed the chloroform based extract kill test organisms at minimum concentration than the others. The least (12.5mg/L) minimum bactericidal concentration was observed in acetone based extract against ATCC *S. aures* and in chloroform based extract against ATCC *K. pneumonia* and *S. enteritidis,* and MDR clinical isolate *P. aeruginosa* while the highest (100mg/L) was observed in all extracts for most of test bacteria specious (**Table 4**).

TABLE 4: MINIMUM INHIBITORY AND MINIMUM BACTERICIDAL CONCENTRATION OF CHILLI PEPPER EXTRACT

MIC/MBC	Extract solvent	MIC/MBC (mg/L)					
		ATCC Bacteria			MDR Clinical isolates Bacteria		
		S. aureus	*K. pneumoniae*	*S. enteritidis*	*P. aeruginosa*	*E .coli*	*S. aureus*
MIC	Acetone	12.5	25	25	25	50	25
	Ethanol	12.5	12.5	25	50	50	25
	Methanol	25	50	25	50	50	25
	Ethyl Acetate	25	50	12.5	12.5	50	12.5
	Chloroform	12.5	12.5	12.5	12.5	100	50
MBC	Acetone	12.5	50	50	50	100	50
	Ethanol	25	25	50	100	50	25
	Methanol	50	100	50	100	100	50
	Ethyl Acetate	50	100	25	50	50	25
	Chloroform	25	12.5	12.5	12.5	100	100

DISCUSSION

Collected Datta samples were characterized physically and microbiologically. The samples had three different colors: red, green, and grey. The difference in color may due to the color of precursor chili pepper which associated with harvesting time. The late harvested chili pepper has red color while the early harvested has green color [4]. In microbial assessment of collected Datta sample, only gram positive rods were observed and these bacteria may be lactobacilli which involved in fermentation of Datta [3, 4]. Lactobacilli had ability to resistant to different food preservatives and diverse intrinsic antimicrobial contents of food.

Datta extracts showed antibacterial activity against all test clinical isolates and standard culture (ATCC) bacteria with zone of inhibition that ranged from 11-27mm. Also along with determination of antimicrobial activities of Datta, antimicrobial activities of chili extract was performed in order to observe if there is significant differences. As information obtained from the Datta collected Hotels, different ingredients such as garlic and ginger were added during preparation and these have also have antimicrobial effect by themselves [13]. From this point of view more antimicrobial activities can expect from Datta as compared to sole Chili Pepper extract. However, our finding showed that there is no significant difference between Datta extract and Chili Pepper extract (P=0.069). This may be due to variation in storage time of both extracts that may be compensated by synergetic effect of ingredients in Datta because the mean storage time of Datta sample was two weeks whereas antimicrobial activities of Chili extract were performed from fresh extracted.

Antimicrobial activities of the Datta and Chili pepper water based extract showed significantly week antimicrobial activities as compared to in cell walls degradation which can extract polar and non-polar phytochemicals. Enzyme polyphenol oxidase is also active in water based extract and this inhibits phenol activities in the extract [14]. Furthermore, alcohols, acetone, ethyl acetate and chloroform are more volatile than water. This property helps them to penetrate the cellular membrane plant materials and extract the intracellular ingredients [15]. Some of phytochemicals that soluble in these solvents include tannins, polyphenols, polyacetylenes, flavone, sterols and alkaloids [16]. In other study methanol extracts of *Capsicum frutescens* were found to be more effective against *S. aureus,* and *Salmonella typhimurium*[7]. Other study also showed ethanol extract has better antimicrobial activities against E. coli and Pseudomonas specious than Chloroform and water extracts [9]. In study conducted by Tsegaye et al, Datta showed antimicrobial activities and eliminates the growth of *E. coli* in the period of fermentation [4]. The other study also showed Datta inhibits Salmonella specious during fermentation period [3]. In another study, on antimicrobial activity of Australian native herb extracts, the aqueous extracts displayed weak antimicrobial activities [17]. Similarly, Weerakkody et al. finding shows that water extracts of black and red pepper had weak antimicrobial activity as compared to ethanol extracts [18].

In this study multi-drug resistant (MDR) clinical bacteria isolate, and ATCC bacteria were used to assess antimicrobial effect of the extracts. Among experimental bacterial species, ATCC® 25923™ *S. aureus* was highly susceptible to extraction solvents while gram negative bacteria *E. coli* of clinical isolate was resistant. This difference may be observed due to the difference in cell wall and cell membrane composition of both groups of bacteria. Study conducted in Iran showed similar finding on *Capsicum annuum* L ethanol extract against clinical isolated microorganisms included of *K. pneumonia, P. aeruginosa, E. coli* and *S. aureus* [8]. In current study the minimum inhibitory concentration and minimum bactericidal concentration supports the finding of agar well diffusion assay. In conducted in Brazil, lower minimum inhibitory concentration was observed against *E. coli, K. pneumoniae,* and *P. aeruginosa*as compared to our study [19]. The observed difference may be due to difference in the amount of active ingredient in a plant can vary with factors like the variety of plant, weather condition of the area, the geographic location, the season and time of harvest, soil conditions, storage conditions, and the method of preparation.

Capsaicin is the main compound in the *Capsicum frutescens* which is responsible for pungency. Chili Peppers contain phenolic compounds, flavonoids and carotenoids [20] which have antimicrobial activity, antibiotic synergism and bacterial virulence removal [21]. In study that isolated phytochemical compound of this specious of Chili Pepper, chrysoeriol was the most active compound that shows high antimicrobial activities [22].

Conclusion: Antimicrobial activities of collected Datta samples have no significant difference among different preparation methods. Significant difference in antimicrobial activity was observed in chili pepper and Datta sample extracted with water from the other solvents. Water was week extractor of active compounds having antimicrobial activities. As a whole standard culture of *S. aureus*was more susceptible organism while standard *S. enteritidis*and clinical isolated MDR *E. coli* were less susceptible for the extracts. There was no significant difference in antimicrobial activity of chili pepper and Datta samples extracts. The traditional use of Datta for food preservation by the local people in various part of Ethiopia is supported by this study.

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