Screening for antibiofilm and antioxidant potential of turmeric (*Curcuma longa*) extracts

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Abstract: The antibiofilm and antioxidant activities associated with turmeric were the main focus of the study. Antibacterial activity was explored against bacteria isolated from dental plaques and dental unit water lines exhibiting resistance against antibiotics and biocides respectively. This study provides a comparison of the natural plant extract against synthetic mouthwash, chemicals and commonly prescribed antibiotics. Methanol extract was more effective as compared to other extracts. Minimum inhibitory concentrations (MIC) ranged from 2.5-10mg/ml. Time based killing kinetic assay showed a significant reduction of bacterial load with increasing concentration of turmeric. Micro titer plate assay indicated significant inhibition of biofilm formation in cells treated with turmeric extract. Phytochemical screening of plant extracts showed the presence of vital secondary metabolites. Flavonoid content and total phenolic content varied among extracts, phenolic content for methanolic extract was 61.669 mg GAE/ gm dry extract and flavonoid content was 3.119mg quercitin/gm dry extract. The values of ferric reducing power were in the range of 5.55- 15.55 mmol of $FeSO_4$ equivalent/ liter of the extract. Antioxidant activities and total phenolic content of the turmeric extracts had significant positive correlation. On the basis of these results turmeric may confidently be recommended as natural antibiofilm and antioxidant agent.

Keywords: *Turmeric, antioxidant activity, antibiofilm activity, total phenolic content.*

INTRODUCTION

Although *Curcuma longa* (known as turmeric) is widely cultivated in South East Asian countries and used as a coloring agent and a spice but many medicinal, antibacterial and antioxidant properties are attributed to this miracle plant (Amman and Wahl, 1991; Luthra *et al.*, 2001). The dried rhizome of turmeric contains a large number of medicinally important phenolic compounds known as the curcuminoids (Lechtenberg *et al.*, 2004). Curcumin is the best studied active ingredient of turmeric and constitutes 0.3-5.4% of crude turmeric (Leung, 1980). The studies indicate that these phenolic compounds may damage cell walls and outer most coverings of bacteria in order to penetrate these cells thereby affecting their metabolic pathways (Marino *et al.*, 2001).

A number of pharmacological properties are attributed to turmeric, it can be used as an antioxidant (Masuda *et al.*, 2002), as an antimicrobial drug (Negi *et al.*, 1999), as antiprotozoal agent (Araujo *et al.*, 1998), as antitumor (Kim *et al.*, 2001), as anti-inflammatory agent (Surh *et al.*, 2001) and anti-allergy agent (Yano *et al.*, 2000). Curcumin, one of the major active compounds isolated from turmeric had shown to possess antibacterial activity against *H. pylori* (Mahady *et al.*, 2002).

Bacterial biofilm can be defined as a community of bacterial cells adhered to biotic and abiotic surface that is encased by a bacterial cellular polymeric matrix. The biofilms help bacteria to survive under harsh environment (Prakash *et al.*, 2003). Colonization of biofilm forming bacteria on dental unit water lines is an emerging concern in the field of dentistry. Moreover, previous studies also explored the resistance of these biofilm formers against biocides used for treatment and cleaning of dental unit water lines (Liaqat and Sabri, 2009). Dental plaque is the biofilm formed by number of microorganisms on the tooth surfaces and its clinical significance is due to resistance exhibited by biofilm formers to various antimicrobial agents (Socrasky and Haffajee, 2002).So there is a need to explore and discover new sources for treatment of biofilm producers in dental unit water lines and dental plaques.

Antioxidants carry importance due to their potential in health promotion and their ability to reduce risk of cancer, heart diseases and hypertension (Wolfe and Liu, 2003; Valko *et al.*, 2007). Herbs and spices are important sources of phenolic compounds (phenolic acid and alcohols, stilbenes, flavonoids, tocopherols), carotenoids and ascorbic acid that show antioxidant activities (Zheng and Wang, 2001).

In this study the antibacterial activity of turmeric crude extract was evaluated against *Bacillus cereus, Bacillus subtilis* and *Klebsiellaspp.* from dental unit water lines (DUWL) and against *Pseudomonasstutzeri, Streptococcus salivarius* and *Erwiniapyrifoliae* from dental plaques by agar well diffusion method. Antibiofilm potential of turmeric extract was determined following micro titre plate assay. Phytochemical screening was performed to

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determine the presence of a number of bioactive compounds in different extracts of turmeric. Total phenolic and flavonoid contents of various extracts of turmeric were also elucidated. Antioxidant activity was done by FRAP (ferric reducing/ antioxidant power) assay. Statistical analysis was done to monitor the relationship between total phenolic content and antioxidant activities of turmeric extracts.

MATERIALS AND METHODS

The rhizomes of turmeric (*Curcuma longa*) were purchased from a local vegetable market in Lahore (Pakistan). These rhizomes were washed with distilled water. All media, reagents and chemicals were obtained from Sigma Chemical Co. (St. Louis, MQ, USA) and Merck (Darmstadt, Germany) and were of analytical grade.

Bacterial strains

Previously isolated and characterized three biocides resistant bacterial strains from dental unit water lines were used in this study. The bacterial strains were *Bacillus cereus* IL2 (DQ989214), *Bacillus subtilis* IL4 (DQ989210) and *Klebsiellaspp.* IL6 (DQ989215). While dental plaques isolates used were *Pseudomonasstutzeri* (KC817808), *Streptococcus salivarius* (KC817807) and *Erwiniapyrifoliae* (KC817810).

Preparation of turmeric extracts

The rhizomes of turmeric were dried and blended in an electric blender to get powdered material. In order to prepare the extracts, 10 grams of powdered turmeric was sequentially extracted with n-hexane, chloroform, ethyl acetate, ethanol, methanol and water. The filtration of extract was done using what man filter paper (No. 1) and the solvent was evaporated by rotary evaporator under reduce pressure at 40^{o}C to get a final concentration of 50 mg/ml of each extract (Garvey *et al.,* 2010).

Phytochemical Screening of the plant extracts

All crude extracts of turmeric (methanol, ethanol, ethyl acetate, chloroform, hexane and aqueous) was analyzed for alkaloids, terpenoids, saponins, tannins, carbohydrates, cardiac glycosides, flavonoids and anthraquinones using the methods of Trease and Evans (1989) and Sofowara (1993).

Antibacterial activity of plant extracts

To evaluate the antagonistic effect, agar well diffusion method was used following Agarry *et al.,* (2005). Accordingly wells of 5 mm diameter were cut on Luria Bertani (LB) agar plates and overnight broth culture of test organisms was spread on these plates. Each of the extracts (0.1 ml) was dispensed into each well and plates were incubated at 37°C for 24 hours. The antagonistic activity was expressed in terms of diameter of zones of the inhibition (mm).

Determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)

To determine minimum inhibitory concentration (MIC), broth dilution method was used (Collins *et al.,* 1995). Two-fold serial dilution of the aqueous extract of turmeric were made with LB broth at final concentrations of 20, 10, 5, 2.5 and 1.25mg/ml. Standardized bacterial culture (absorbance of initial inocula adjusted to 0.1A at 600 nm) was inoculated (0.1ml) into the dilutions and incubation was done at 37^{o}C for 24 hours. The lowest concentrations which showed no turbidity was recorded as the MIC of the extracts.

Minimum bactericidal concentration (MBC) was also monitored to find out whether the bacterial strains were actually killed or their growth was merely inhibited. For this, the content of the tubes showing MIC and tubes with concentrations greater than MIC were spread on separate LB agar plates and incubated at 37^{o}C for 24hours. The lowest concentration showing no growth on the plate was recorded as MBC.

Time to kill kinetic assay

The rate of kill of turmeric extract was determined by using the method of Okoli and Iroegbu (2005). For this, 0.1 ml of standardized culture (absorbance of initial inocula adjusted to 0.1A at 600nm) was added into LB medium supplemented with plant extracts at concentrations of MIC, 2 × MIC, 4 × MIC. After incubation at 37^{o}C for 24hours, 0.1 ml of sample was removed from cultures at 0,1, 2, 3, 4, 5, 6 and 24 hours. After serial dilutions, 0.1 ml of diluted sample was plated on LB agar plates. The plates were incubated at 37^{o}C for 24hours. Controls included extract free broth that with inoculation of respective bacterial strain.

Effect of turmeric extract on biofilm formation

Effect of the aqueous turmeric extract on biofilm formation was monitored following the method of Christensen *et al.,* (1985) micro titer plate assay with necessary modifications. For this purpose sub-inhibitory concentrations (1/2 × MIC) of turmeric extract were added to the wells having bacterial cultures inoculated in LB broth. Negative and positive controls were also included in the experiment using only sterile growth medium and working solution, respectively. Experiments were carried out in replicate. Plates were incubated at 37°C for 72, 120 and 175 hours. After incubation the growth medium was discarded, and the wells were washed thrice with sterile physiological saline (0.85% NaCl). The adhered cells were stained with 0.1% crystal violet for 10 min. The excessive stain was removed by washing twice with 0.85% NaCl, while cell-bound dye was eluted with 200µl of 33% glacial acetic acid and the absorbance of eluted solution was measured at 578 nm using a micro titer plate reader.

Total phenolic content (TPC) determination

All extracts of turmeric (ethanol, chloroform, methanol, hexane, ethyl acetate and aqueous extracts) were analyzed for total phenolic compounds. It was estimated through Folin-Ciocalteuassay with slight modifications (Zhou and Yu, 2006). For this purpose, 1ml of the extract, 0.5ml of the Folin-Ciocalteu reagent, 1ml of 10g/100ml sodium carbonate and 7.5 ml of distilled water were mixed. The tubes were left at room temperature for 45 minutes, and then absorbance was measured at 765 nm by using a UV-visible spectrophotometer. Gallic acid was used as standard (at concentrations ranging from 0 to 500µg/ml, r^2= 0.993). The results were expressed as milligrams of gallic acid equivalent (GAE) per one gram of dry extract.

Flavonoid content determination

In order to determine flavonoid content, method of Chang *et al.,* (2002) was followed with slight modifications. Each plant extract was mixed separately with 1.5ml of methanol, 0.1 ml of 1M potassium acetate, 0.1ml of 10% aluminium chloride and 2.8ml of distilled water. The reaction mixture was left at room temperature for 30 minutes and absorbance was measured at 415nm. Quercitin was used as a standard at concentrations of5 to 100µg/ml, r^2=0.963. The results were presented as mg quercitin/ gram dry extract.

Ferric reducing/antioxidant power (FRAP) assay

FRAP assay was done following Benzie and Strain (1996) method with slight modifications. Briefly, 85µl of plant extract was mixed with2750µlof diluted FRAP reagent (1:1 with distilled water) and this mixture was incubated at37^{0}C for 4 min. The absorbance was monitored at 593 nm using distilled water as a blank.$FeSO_4.7H_2O$ (0.5-5 mmol/L) was used for standard curve. The results were presented as mmol $FeSO_4$ equivalents per liter of the extract.

STATISTICAL ANALYSIS

The experiments were performed in replicates and their results were described as mean and standard error (S.E). Significant differences were calculated using Student's t-test and Duncan's multiple range tests whereas correlations were determined by Pearson correlation coefficient. Level of significance was determined at P< 0.05 (Steel and Torrie, 1981).

RESULTS

Phytochemical screening showed presence of a number of bioactive compounds such as alkaloids, anthraquinones, glycosides, flavonoids, phenolics and terpenoids while secondary metabolites such as saponins and tanins were absent in turmeric (table 1).

The results indicated that the antibacterial property of turmeric extracted by using different solvents. Varying antimicrobial response towards the bacterial isolates used in this study (table 2). Methanolic and ethanolic extracts showed the best bioactivity against *B. cereus*. While crude extract of turmeric, extracted in ethyl acetate, was best against *B. subtilis*. In most of the cases, different extracts exhibited more inhibitory potential against Gram positive bacteria compared to the Gram-negative bacteria. Crude extract prepared in hexane extract did not show inhibition against any strain while for chloroform extract associated inhibition was observed only against *Klebsiella* sp. (table 2).

Table 1: Screening of phytochemical constituents of different extracts of turmeric.

Phytochemicals	Present/ Absent
Alkaloids	+
Anthraquinones	+
Cardiac glycosides	+
Flavonoids	+
Phenolics	+
Phalobatanins	_
Saponins	_
Tanins	_
Terpenoids	+

The results of minimum inhibitory concentrations (MIC) and minimum bactericidal concentrations (MBC) showed that MIC and MBC for Gram positive and Gram-negative strains were different from one another. These results indicated that turmeric extract had the ability to inhibit and completely kill the strains used in this study (table 3).

Turmeric also showed bactericidal behavior as indicated by rate of killing of bacteria in time kill kinetic assay. Reduction in bacterial load was observed as concentration of turmeric was increased in growth media. After 5 hours of incubation at MIC and after 3 hours at 2 × MIC and 4 × MIC CFU per ml was found to be zero in turmeric treated cells of *B. cereus* and *B. subtilis*. While in case of *Klebsiella* different results were recorded. At MIC after 24 hours, at 2 × MIC after 6 hours and at 4 × MIC after 5 hours CFU per ml was decreased (fig. 1). Whereas in case of *P. stutzeri* (isolated from dental plaques) at MIC, after 6 hours CFU per ml was found to be zero. In case of *E. pyrifoliae* and *S. salivarius*, after 5 hours, CFU per ml was found to be zero when turmeric was added at minimum inhibitory concentration (fig. 2).

To check the effect of turmeric extract on biofilm formation, micro titer plate assay was used. The results had indicated that turmeric significantly inhibited biofilm formation in bacterial cells as compared to the control cells ($p<0.05$). It was also observed that biofilm formation was significantly reduced after 175 hours of incubation as compared to 72 hours of incubation as indicated by a significant decrease in optical density measured at 578

nm. The results also showed that in case of *Bacillus subtilus* and *Klebsiella* sp., biofilms were well established after 120 hours of incubation and then cells were removed as indicated by adecrease in optical density at 175 hours of incubation. In case of *E. Pyrifoliae* maximum biofilm formation was observed after 175 hours. However, the presence of turmeric extract significantly reduced biofilm formation in strains isolated from dental plaques (fig. 4).

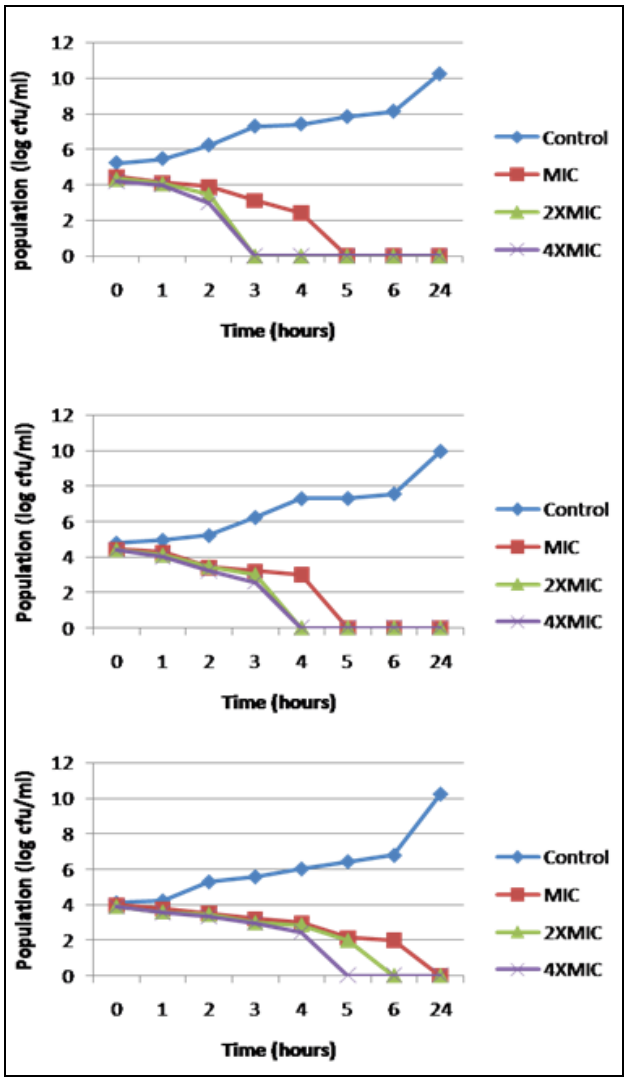

Fig. 1: Total viable count of bacterial strains isolated from dental unit water lines in LB medium supplemented with different conc. of turmeric extract. (a)*B. cereus*, (b) *B. subtilus*, (c) *Klebsiella* sp.

Determination of total phenolic content indicated that different extracts of turmeric showed different concentrations of total phenolic content (table 4). It is clear from the results that the highest amount of phenolic was observed for ethanol extract followed by methanol extract with mean value of 61.669 mg GAE/ gm of extract and 60.635 mg GAE/ gm of extract respectively. The results also exhibited a significant difference ($p<0.05$) among mean values of total phenolic contents of extracts studied except for ethyl acetate and aqueous extracts (table 4).

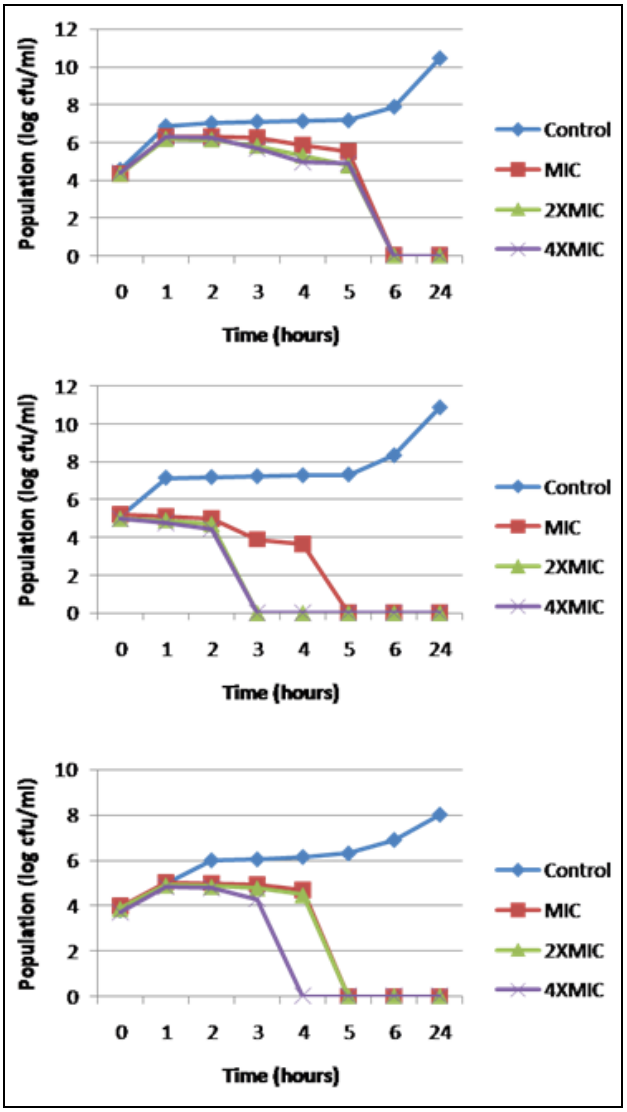

Fig. 2: Total viable count of bacterial strains isolated from dental plaques in LB medium supplemented with different conc. of turmeric extract. (a) *P. stutzeri*, (b) *E. pyrifoliae*, (c) *S. salivarius*.

For the determination of total flavonoid content, quercitin was used as a calibrating standard. The results showed that methanolic extract had more flavonoid compared to others in order of: methanol > ethanol > hexane > chloroform > aqueous > ethyl acetate. It was also observed that in some cases turmeric extracts exhibited significant differences ($p<0.05$) in flavonoid content when compared with each other (table 5).

FRAP assay (used to measure antioxidant activity) showed great variation among all the variants of crude extracts studied. Methanol extract showed the highest FRAP activity followed by ethanol extract, while

minimum FRAP activity was observed for hexane extract. Antioxidant activity of methanol extract was significantly different from other extracts except for ethanol extract (table 6). A positive correlation was recorded between antioxidant activity and total phenolic content (r = 0.90), while a negative correlation was observed between antioxidant activity and to flavonoid content (r = 0.439).

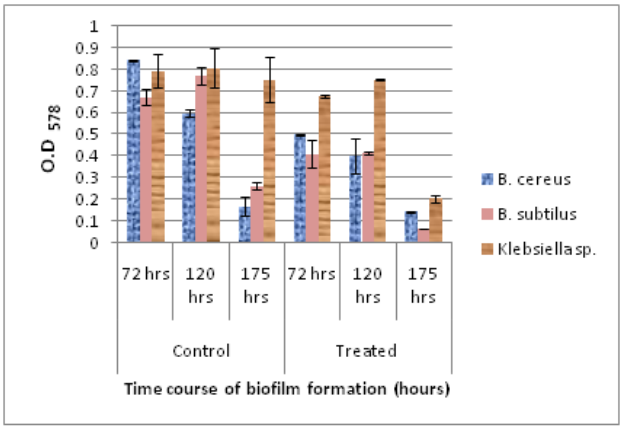

Fig. 3: Effect of turmeric extract on biofilm formation at different time intervals (72, 120 and 175 hours) of strains isolated from dental unit water lines.

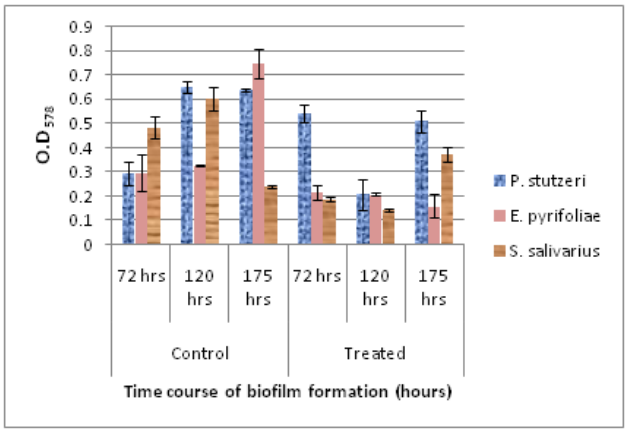

Fig. 4: Effect of turmeric extract on biofilm formation at different time intervals (72, 120 and 175 hours) of strains isolated from dental plaques.

DISCUSSION

Plants are considered as vital source of pharmacologically active ingredients for a long period of time. When plants and their phytochemical constituents having known antimicrobial and antioxidant properties are used in therapeutic treatments, they show significant results (Gislene *et al.*, 2000). Turmeric is not only a medicinal plant but it is also an efficient home remedy to cure various disorders (Hitesh *et al.*, 2011). The studies had indicated that pharmacological attributes of turmeric are due to its vital constituent known as curcumin, which has been widely researched for its antimicrobial, anti-inflammatory, antioxidant, anti-angiogenic and anti-cancer activities (Jayaprakasha *et al.*, 2006).

The present study indicated significant antibacterial activity of different extracts of turmeric against the target bacterial strains as observed in agar well diffusion method and time kill kinetic assay. It is evident that antimicrobial activities of turmeric are largely due to the presence of alkaloids, essential oils, curcumins, turmeric oil, tumerol and veleric acid (Cikrikci *et al.*, 2008). One of the studies also showed that curcumin and various other oil fractions found in turmeric had affected the growth of *Pseudomonas aeruginosa* by down regulating the expression of virulence factors, biofilm initiation and quorum sensing (Rudrappa and Bais, 2008). Earlier studies indicated that bioactive compounds of plant extracts may show their antibacterial effect by acting on various target sites such as by degradation of bacterial cell wall, by damaging cytoplasmic membrane, by damaging membrane proteins, leakage of various cell constituents and by depleting proton motive force (Burt, 2004). Thus turmeric extracts and their phytochemical constituents may act on any of the above mentioned target sites. The present study indicated that maximum inhibition by turmeric was directed against *B. Cereus* strain (diameter of zone of inhibition was 17 mm). It was observed in a study by (Negi *et al.*, 1999) that curlone and tumerone found in turmeric exhibited excellent antagonistic activity against the bacterial strains such as *P. aeruginosa*, *B. subtilis*, *B. cereus*, *B. coagulans*, *E. coli* and *S. aureus*.

The results of present study also indicated the antibiofilm activity of aqueous turmeric extract by reducing the ability of bacteria to adhere to polystyrene surface in micro titer plate assay as indicated by decrease in absorbance as compared to the untreated cells. Previous studies also indicated antibacterial activity of turmeric by inhibiting biofilm formation. One of the studies by (Pattiyathanee *et al.*, 2009) clearly indicated that biofilm formation by *Helicobacter pylori* was significantly reduced at sub-inhibitory concentrations of curcumin (one of the active ingredients of turmeric). However, according to some studies the turmeric extracts did not significantly affect biofilm formation or even no effect was observed. One of the previous studies indicated that among all the herbs studied for anti-adhesive effect, only turmeric was not found to provide significant results as compared to other herbs (Barreto *et al.*, 2001).

Numerous studies had indicated that herbs and spices exhibited significant antioxidant activities largely due to the presence of large number of phenolic compounds (Wu *et al.*, 2006; Wong *et al.*, 2006). Our study also indicated the presence of phenolic compounds in different extracts of turmeric as evidenced by phenolic content of the extracts. It was reported that reducing power of plant extract was associated with its antioxidant activity as

Table 2: Zones of Inhibition (in mm) by turmeric extracted (with various solvents).

Strains	Diameter of zones of inhibition (mm) for extracts used				
	Methanol	Ethanol	Ethyl acetate	Chloroform	Aqueous
B. cereus	17±0.707	11±0.707	10±0.707	0	5±0.353
B. subtilis	12±0.707	8±1.060	14±1.414	0	7±0.707
Klebsiella sp.	15±0.353	6±0	7±0	7.5±0.353	4±0
P. stutzeri	6.5±0.707	9±0	11±0.353	0	7±0
E. pyrifoliae	2±0.707	0	3±0.353	3±0	3±0.707
S. salivarius	5±0.353	4±0	3±0.707	3±0	0

All the data are expressed as mean ± standard deviation.

evidenced by the activity of anthraquinones (Yen *et al.*, 2000; Yen and Duh, 1993). In the present study plant extracts exhibited different response towards reducing power as indicated by variation in their ferric reducing power (FRAP) values.

Table 3: MIC and MBC (mg/ml) values of turmeric extract against tested bacteria

Strains	MIC (mg/ml)	MBC (mg/ml)
B. cereus	5	10
B. subtilis	10	10
Klebsiella sp.	10	20
P. stutzeri	5	10
E. pyrifoliae	5	10
S. salivarius	2.5	5

Table 4: Total phenolic content of turmeric extracted with different solvents

Plant Extracts Used	Conc. mg GAE/gm dry plant
Methanol	60.635 ± 0.03^{d}
Ethanol	61.669 ± 0.08^{e}
Ethyl acetate	58.441 ± 0.435^{c}
Chloroform	55.341 ± 0.01^{a}
Hexane	57.265 ± 0.016^{b}
Aqueous	59.143 ± 0.434^{c}

All data are expressed as mean ± standard error.

Table 5: Flavonoid content of turmeric extracted with different solvents.

Plant extracts used	Conc. mg quercitin/gm dry plant
Methanol	3.119 ± 0.01^{d}
Ethanol	$2.966\pm0.04^{c,d}$
Ethyl acetate	2.205 ± 0.098^{a}
Chloroform	2.512 ± 0.055^{b}
Hexane	2.857 ± 0.055^{c}
Aqueous	2.282 ± 0.042^{a}

All data are expressed as mean ± standard error.

The phenolic compounds can show a significant correlation with the antioxidant activities of turmeric extracts as observed by positive correlation (r=0.90) between phenolic contents and antioxidant activities evidenced by ferric reducing power assay. One of the previous studies had indicated that curcumin (a phenolic compound found in turmeric) exhibited strong antioxidant activities (Miquel *et al.*, 2002). A negative correlation between antioxidant activities and flavonoid content was observed in the present study, but earlier studies had indicated that flavonoids present in the rhizomes of *Curcuma amada* were mainly responsible for its antioxidant activity (Policegoudra *et al.*, 2007).

Table 6: Ferric reducing power of turmeric extracted with different solvents.

Plant Extracts Used	Mmol of $FeSO_4$ equivalent/ litre of plant extract)
Methanol	15.55 ± 0.25^{d}
Ethanol	13.7 ± 0.2^{d}
Ethyl acetate	8.65 ± 0.25^{b}
Chloroform	6.2 ± 0.3^{a}
Hexane	5.55 ± 0.35^{a}
Aqueous	11.6 ± 1.2^{c}

All data are expressed as mean ± standard error.

It can be concluded that turmeric extracts showed variations in their antibiofilm and antioxidant activities. Our study showed the presence of large number of bioactive compounds. A further study is required to purify bioactive compounds so that actual mode of their antibacterial and antioxidant activities can be elucidated in future.

REFERENCES

Agarry OO, Olaleye MT and Bello-Michael CO (2005). Comparative antimicrobial activities of *Aloe vera* gel and leaf. *Afr. J. Biotechnol.*, **4**(12): 1413-1414.

Ammon HP and Wahl MA (1991). Pharmacology of *Curcuma longa*. *Planta Med.*, **57**: 1-7.

Araujo CAC, Alegio LV, Castro D, Lima MEF and Leon LL (1998). *Leishmania amazonensis*: *In vivo* experiments with diarylheptanoids from Leguminosae and Zingiberaceae plants. *J. Memorias doInstituto Oswaldo Cruz.*, **93**: 306-310.

Barreto G, Campal AC, Abreu O and Velásquez B (2001a). El bloqueo de la adhesion Fimbrialco-moopciónterapéutica. *Rev. Prod. Anim.,* **13**(1): 71-82.

Benzie IFF and Strain JJ (1999). Ferric reducing /antioxidant power assay: Direct measure of total antioxidant activity of biological fluids and modified version for simultaneous measurement of total antioxidant power and ascorbic acid concentration. *Meth. Enzymol.,* **299**: 15-27.

Burt S (2004). Essential oils: Their antibacterial properties and potential applications in foods-a review. *Int. J. Food Microbiol.,* **94**: 223-253.

Chang C, Yang M, Wen H and Chem J (2002). Estimation of total flavonoid content in propolis by two complementary colorimetric methods. *J. Food Drug Anal.,* **10**: 178-182.

Christensen GD, Simpson WA, Younger JJ, Baddour LM, Barrett FF, Melton DM and Beachey EH (1985). Adherence of coagulase negative *staphylococci* to plastic tissue culture plates: A quantitive model for the adherence of *staphylococci* to medical devices. *J. Clin. Microbiol.,* **22**: 996-1006.

Collins, GH, Lynes PM and Grange JM (1995). Microbiological Methods. 7th edition. Butterwort-Heinemann Ltd, Britain. Pp.175-190.

Cikricki S, Mozioglu E and Yylmaz H (2008). Biological activity of curcuminoids isolated from *Curcuma longa*. *Rec. Nat. Prod.,* **2**: 19-24.

Gislene GF, Nascimento JL, Paulo CF and Giuliana LS (2000). Antibacterial activity of plant extracts and phytochemicals on antibiotic resistant bacteria. *Braz. J. Microbiol.,* **31**: 247-256.

Hitesh K Dhamija and Ankit S Chauhan (2011). Herbs: Now in arena of hyperlipidaemia. *Der. Pharmacia Sinica.,* **2**(3): 51-59.

Jayaprakasha GK, Jaganathan Rao L and Sakariah KK (2006). Antioxidant activities of curcumin, demethoxycurcumin and bisdemethoxycurcumin. *Food Chem.,* **98**: 720-724.

Kim MS, Kang HJ and Moon A (2001). Inhibition of invasion and induction of apoptosis by curcumin in H-ras-transformed MCF-10A human breast epithelial cells. *Arch. Pharm. Res.,* **24**: 349-354.

Lechtenberg L, Quandt B and Nahrstadt A (2004). Quantitative determination of curcuminoids in Curcuma rhizomes and rapid differentiation of *Curcuma domestica* Val. And *Curcumazanthorrhiza* Roxb by capillary electrophoresis, *Phytochem. Anal*., **15**: 152-158.

Leung A (1980). Encyclopedia of common natural ingredients used in food, drugs and cosmetics. New York, NY. John Wiley. pp.313-314.

Liaqat I and Sabri AN (2009). Isolation and characterization of biocides resistant bacteria from dental unit water line biofilms. *J. Basic Microbiol.,* **49**: 275-284.

Luthra PM, Singh R and Chandra R (2001). Therapeutic uses of *Curcuma longa* (Turmeric). *Indian J. Clinical Biochem.,* **16**: 153-160.

Mahady GB, Pendland SL, Yun G and Lu ZZ (2002). Turmeric (*Curcuma longa*) and curcumin inhibit the growth of *Helicobacter pylori*, a group 1 carcinogen. *Anticancer Res.,* **22**: 4179-4181.

Marino M, Bersani C and Comi G (2001). Impedance measurement to study antimicrobial activity of essential oils from *Lamiaceae* and *Compositae*. *Int. J. Food Microbiol.,* **67**: 187-195.

Mark GI, Rahman M, Gibbons S and Piddock LJV (2011). Medicinal plant extracts with efflux inhibitory activity against Gram-negative bacteria. *Int. J. Antimicrob. Agents.,* **37**(2): 145.

Masuda T, Toi Y, Bando H, Maekawa T, Takeda Y and Yamaguchi H (2002). Structural identification of new curcumin dimers and their contribution to the antioxidant mechanism of curcumin. *J. Agric. Food Chem.,* **50**: 2524-2530.

Miquel J, Bernd A, Sempere JM, Diaz-Alperi and Ramiraz A (2002). The curcuma antioxidants: Pharmacological effects and prospects for future clinical use. *A review. J. Microbes Infect,* **2**: 125-129.

Negi PS,Jayaprakasha, Jagan Mohan Rao L and Sakariah KK (1999). Antimicrobial activity of turmeric oil: A by-product from curcumin manufacture. *J. Agric. Food Chem.,* **47**: 4297-4300.

Okoli S and Iroegbu CU (2005). *In vitro* antibacterial activity of *Synclisascabrida* whole root extracts. *Afr. J. Biotechnol.,* **4**(9): 946-952.

Pattiyathanee P, Vilaichone R and Chaichanawongsaroj N (2009). Effect of curcumin on *Helicobacter pylori* biofilm formation. *Afr. J. Biotechnol.,* **8**(19): 5106-5115.

Policegoudra RS, Abiraj K, Gowdab DC and Aradhya SM (2007). Isolation and characterization of antioxidant and antibacterial compound from mango ginger (*Curcuma amada* Roxb.) rhizome. *J. Chromat.,* **852**: 40-48.

Prakash B, Veeregowda BM and Krishnappa G (2003). Biofilms: A survival strategy of bacteria, *Curr. Sci. Rev.,* **85**: 1299-1307.

Rudrappa T and Bais HP (2008). Curcumin, a known phenolic from *Curcuma longa*, attenuates the virulence of *Pseudomonas aeruginosa* PAO1 in whole plant and animal pathogenicity models. *J. Agric. Food Chem.,*26: 1955-1962.

Socransky SS and Haffajee AD (2002). Dental biofilms: Difficult therapeutic targets. *Periodontol.,* **28**: 12-55.

Sofowara AE (1993). Medicinal plants and traditional medicine in Africa. Vol. 2. Spectrum Books Ltd, Ibadan, p.288.

Steel RGD and Torrie JH (1981). *In*: Principles and procedures of statistics, a biochemical approach (2nd Edition). McGraw Hill International Book Company.

Surh YJ, Chun KS, Cha HH, Han SS, Keum YS and Park KS (2001). Molecular mechanisms underlying chemo preventive activities of anti-inflammatory phytochemicals: Downregulation of COX-2 and iNOS through suppression of NF-Kappa B activation. *Mutation Res.,* **480**: 243-268.

Trease GE and Evans WC (1989). Pharmacology 11th Ed., Bailliere Tindall Ltd., London, pp.60-75.

Valko M, Leibfritz D, Moncola J, Cronin MTD, Mazura M and Telser J (2007). Free radicals and antioxidants in normal physiological functions and human disease. *Int. J. Biochem. Cell Biol.,* **39**: 44-84.

Wolfe KWX and Liu RH (2003). Antioxidant activity of apple peels. *J. Agric. Food Chem.,* **51**(3): 609-614.

Wong C, Li H, Cheng K and Chen F (2006). A systematic survey of antioxidant activity of 30 Chinese medicinal plants using the ferric reducing antioxidant power assay. *Food Chem.,* **97**: 705-711.

Wu CQ, Chen F, Wang X, Kim HJ, He GQ, Haley-Zitlin V and Huang G (2006). Antioxidant constituents in feverfew (*Tanacetumparthenium*) extract and their chromatographic quantification. *Food Chem.,* **96**: 220-227.

Yano S, Terai M, Shimizu KL, Sekine T, Yamamoto Y, Takamoto K, Saito K, Ueno K and Watanabe K (2000). Antiallergic activity of *Curcuma longa* (I) Effectiveness of extracts containing curcuminoids. *Nat. Med.,* **54**: 318-324.

Yen GC and Duh PD (1993). Antioxidant properties of methonolic extracts from peanut hull. *J. Am. Oil Chem. Soc.,* **70**: 383-386.

Yen GC, Duh PD and Chuang DY (2000). Antioxidant activity of anthraquinones and anthrone. *Food Chem.,* **70**: 437-441.

Zheng W and Wang S (2001). Antioxidant activity and phenolic composition in selected herbs. *J. Agric. Food Chem.,* **49**: 5165-5170.

Zhou K and Yu L (2006). Total phenolic contents and antioxidant properties of commonly consumed vegetables grown in Colorado. *Lebensmittel-Wissenschaft und Technologie.,* **39**: 1155-1162.