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Research

Therapeutic effects of Lemon Grass (*Cymbopogon citratus)* against clinically important pathogens

Nighat Zia ud Den[1*], Muhammad Shahid[2], Mudasir Majeed[3],Umar Daraz Khan[4], Amer Jamil[5], Sabah Javaid[6], Muhammad Imran Kanjal[7]

[1]*Department of Biochemistry, University of Agriculture, Faisalabad.*

[2]*Department of Biochemistry, University of Agriculture, Faisalabad.*

[3]*Department of Applied Chemistry, Government College University, Faisalabad.*

[4]*Department of Veterinary Sciences, University of Agriculture, Faisalabad.*

[5]*Department of Biochemistry, University of Agriculture, Faisalabad.*

[6]*Department of Biochemistry, University of Agriculture, Faisalabad.*

[7]*Department of Chemistry, Government College University, Faisalabad.*

[*]***Correspondence author***

Email ID; nighatzia16@gmail.com

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Abstract: *Globally, resistance to antibiotics has become a big concern and has a significant effect on the health of patients. Effective treatment approached must be explored in order to resolve this problem. Lemon grass (Cymbopogon citratus) is an essential medicinal plant with numerous biological and pharmacological potential that is helpful towards infectious diseases susceptible to multi-drugs.Therefore, present study was conducted to evaluate the antioxidant potential and antimicrobial activities of different extracts of Lemon Grass. Antioxidant potential of the plant was evaluated by different antioxidant parameters. Multidrug resistant gram positive and negative bacteria were collected from microbiology department. Comparative analysis of lemon grass was performed among aqueous, methanolic and aqueous extracts treated with different temperatures. Study results indicated that highest total phenolic contents were found in lemon grass extract treated at 170ºC while the least contents were found in methanolic extract. Due to higher contents of phenolic and flavonoid compounds, lemon grass exhibited good antioxidant potential by scavenging the free radicals and through reducing power assay. From the results it was found that the extracts of lemon grass have antimicrobial activity (>0.51 activity index) against tested Gram negative and Gram positive bacterial strains. The study concluded that lemon grass has therapeutic potential due to the presence of antioxidant compounds and its antimicrobial activity that makes it a potential source of medicines. Further*

studies are required to explore its healing properties against various disorders through its biological activities and characterization of bioactive compounds present in it.

Key words: *Cymbopogon citrate*, Antioxidant assays, Antimicrobial assay, Total phenolics, Total Flavonoids

Introduction

Cymbopogon citratus is a therapeutic herb belongs to family *poaceae* found in India and is established in sub-tropical and tropical regions. It is frequently utilized as a medicinal drug. Its oil is used as insecticide and has protective effect against certain fungal infections (Deepa *et al.*, 2012). In addition, it can also be used as antipyretic agent. The extract of lemongrass leaves can be used as drink which is useful in reducing the belly fat as well as stomach pain (Isam *et al*., 2009).

Due to the presence of flavonoid contents such as myrcene, geraniol, citronellol and citronellal, lemon grass plays a significant role in muscle relaxation and relaxation from mental stress as reported by Anibijuwon (2010). Many studies were conducted on the lemongrass to show that it exhibits various biological activities including anticancer activity and antioxidant activity. Extract of lemon grass have no toxic effects against tested microorganisms but in fact, it helps to reduce the growth of infected microorganisms. Lemon grass tea or infusion use is popular as medicine in many countries. It is prepared with fresh and dried leaves and covers a wide range of indications (Behboud *et al*., 2012).Due to presence of its active chemical constituent (citral), it is normally being used for digestion as well as for muscle relaxation (Rathabai, 2013). *Cymbopogon citratus* have unique properties as a carminative agent and functions as insect repellant. Alkaloid, saponins and tannins are the most important bio-active components of this plant (Elodie *et al.*, 2013; Abd ul Fattah, *et al.*, 2010; Adegbegi *et al*., 2012).

Reactive oxygen species and volatile compounds such as superoxide radicals and OH^- radical species that are involved in cellular activities including regulation of cell proliferation and phagocytosis. These scavenging radicals interact with oxidative reactions that help to prevent oxidative stress induced by either external factors or internal factors.

Multidrug-resistant bacteria are a serious problem which has rapidly spread worldwide. Although multidrug bacterial infections are currently being treated using antibiotics (Borneo *et al.*, 2009; Netzel *et al*., 2007). However, some first and second line antibiotics are rapidly becoming ineffective in the treatment of multidrug bacterial infections due to the emergence of antibiotic

resistance (Ventola, 2015; Lin et al., 2015). Natural alternative treatment could be an option to help overcome multidrug resistant bacterial infections and as source of strong antioxidant potential. Thus, the objective of present study was find the efficacy of aqueous, methanolic and boiled extracts at different temperature of lemon grass as an antimicrobial agent and strong antioxidant potential source.

Material and Methods

Collection of plant material

Cymbopogan citratus (Lemongrass) was collected from the botanical garden of University of Agriculture Faisalabad. The aerial part of the plant was washed and dried at room temperature and ground it into fine powder for extraction.

Aqueous and methanolic extraction

The collected aerial parts of plant were washed, shade dried and then grounded into fine powder. The plant material was extracted in aqueous and methanolic solvent by dissolving powdered plant material in distilled water and methanol (1:10 w/v) in sterile flask and placed on orbital shaker for 3 days at 10,000 rpm. After 3 days, the extracts were concentrated by using rotatory evaporator and stored in a refrigerator at 4 ºC for further analysis (Archana and Jayanthi, 2011, Tariq and Reyaz, 2012).

Preparation of lemon grass (*Cymbopogan citratus*) extract at different temperature

The extract (aqueous) of lemongrass was further processed at two different temperatures (120 ºC and 170 ºC) to make decoction and change in temperature was measured by using thermometer.

Antioxidant parameters

Total Phenolic Contents (TPC) and Total Flavonoid Contents (TFC)

Total phenolic contents were measured using Folin- Ciocalteu reagent as by using the method as described by Ainsworth and Gillespie (2007) with slight modifications. In this method sample extract (100µL) was added with (10% diluted) Folin Ciocalteu reagent (200 µL) and vortexe thoroughly. After that added Na_2CO_3 (800 µL of 700mM) into each sample and incubated at room temperature for 2 hours and absorbance was taken at 765 nm. Gallic acid was used as standard and the results were expressed as Gallic acid (mg/g) equivalent (GAE) per dry matter.

Total flavonoid contents were measured by using the method described by Dewant *et al.,* (2002) with slight modifications. Sample extract (1mL) was taken in test tube and then added 5%

$NaNO_2$ (3mL), 0.6mL of $AlCl_3$ (10%) was added after 5 minutes and 2mL of NaOH (1M). Absorbance was taken at 510 nm. Catechin (mg/g) per dry matter used as standard.

DPPH radical scavenging assay

Sample (50µL) was added in 5 mL of (0.004 %) DPPH methanol solution. The absorbance was measured at 517 nm after the incubation for 30 minutes at room temperature.

$$I\ \% = (A_{blank} - A_{sample} / A_{blank}) * 100$$

In which A_{blank} is the absorbance of DPPH solution and A_{sample} being the absorbance of sample (Bozin *et al.,* 2006).

Assessment of reducing power activity

The reducing power of the extracted samples was calculated by the method expressed by the Yen *et al.,* (2000) with slight modification. 5 mL of (0.2 M) potassium phosphate buffer (pH,6.6), 5 mL of potassium ferricyanide (1%) was mixed with 100 µL of the sample and was incubated for 20 minutes at 37 ºC and centrifuged for 10 minutes at 10,000 rpm and then added 5mL of (10 %) trichloroacetic acid and 1 mL of (0.1 M) ferric chloride. Absorbance was measured at 700 nm.

$$\text{Increase in reducing power (\%)} = Abs_{sample} - Abs_{blank} / Abs_{blank} * 100$$

Antimicrobial assay

Antimicrobial activity of aqueous and methanolic extracts was determined against selected Gram negative (*E.coli, P.multicoida* and *A. umefaciens*) and Gram positive (*B.subtilis* and *S.aureus*) bacterial species (CLSI, 2007). Microbial strains selected for this study includes; *Bacillus subtilis, Staphylococcus aureus, Escherichia coli, Pasteurella multocida, and Agrobacterium tumefaciens*.

Anti-Bacterial assay by Disc diffusion method

Nutrient agar (28.08 g/L) was dissolved in distilled water and autoclaved at 121 ºC for 15 min. After sterilization, media was poured in sterilized petri plates and were subjected to bacterial species when media slightly cool down at room temperature. Distilled water and chloramphenicol were used as negative and positive control, respectively. Samples solution was poured on sterilized disc made form whatsmann filter paper No 1 and these discs placed on solidified media poured inside the petri plates. The extract having antibacterial activity showed

clear zone of inhibition radius were measured by zone reader in millimeter (Banerjee *et al.*, 2014).

Minimum Inhibitory Concentrations (MIC) of plant extracts

Nutrient broth (100µL) was sterilized by using autoclave and when it became cool then poured in microtiter plates. Then 100µL of tested sample was poured in each well of microtiter plate and dilute it by using two-fold serial dilution method. After that, 20 µL bacterial strain was added in all wells and then it was placed over night for incubation at 37 ºC. Resurizine was used as indicator which changed the color of well into purple or pink due to growth of bacteria and the absorbance was measured at 597 nm.

Hydrolysis of biofilm formation

The method described by the Dheepa *et al*. (2011) was used for the quantitative measurement of hydrolysis of biofilm formation. Take 100 µL nutrient broth, test sample and 10 µL bacterial culture in each well of microtiter plate and incubated overnight at 37 ˚C. Next day washing was done by distilled water and fixation of biofilm done by methanol. Then staining was done by 2 % crystal violet. The appearance of adherent film of strained material on the inner surface of the wells showed the positive results. The wells which contain nutrient broth were considered as negative control. Hydrolysis of biofilm was done by (33%) glacial acetic acid solution and absorbance was measured at 630 nm. Percentage hydrolysis of biofilm was measured by the formula.

$$\%\text{age of INH} = 100 - \left(\frac{\text{OD of sample}}{\text{OD of positive control}}\right) \times 100$$

Where OD of sample was optical density of sample measured by Elisa reader and OD of positive control means optical density of sample without bacterial culture.

Statistical evaluation

Two-way ANOVA and LSD were applied by using Minitab version 10 to evaluate the significant results among the solvents used (Arora *et al.*, 2009).

Results and Discussion

Antioxidant activity of Lemon Grass

Total phenolic contents (TPC) and Total flavonoid contents (TFC)

Having one or more hydroxyl group, the phenolic groups were unique and greatest compounds as medicinal plant metabolites. Phenols and polyphenols practice their defensive affects for various ailments including cancers (Claudine *et al.*, 2004).

From the Table 1, it was found that extract of lemongrass at 120 ˚C and 170 ˚C have high phenolic contents (1.25±0.10 and 1.08±0.24 mg/g) while aqueous extract of has (0.37±0.03 mg/g) and methanolic extract (0.23±0.02 mg/g). These results indicate that treatment at different temperature 120 ˚C and 170 ˚C significantly have high phenolic contents as compared to other solvents. This is because temperature treatment helps to scavenge the free radicals more actively. From the above mention results it was concluded that extracts of lemongrass at different temperatures have high phenolic contents as compared to aqueous and methanolic extracts of lemongrass. The antioxidant activity indicates that the plant has significant concentrations of phenolic and flavonoid compounds. Previous studies reported anticancer agents in lemongrass which can be extracted more efficiently at different temperature especially treatment at 120˚C (Saeedeh and urooj, 2007). Antioxidant activity of phenolic compounds mainly due to their redox potential can plays an important role in absorbing and neutralizing the free radicals, quenching the singlet and triplet oxygen or decomposing peroxides (Tomczyk and Pleszezynska, 2010).

Results of comparative analysis of lemongrass extracts were given in Table 1 which indicated that extract at different temperature treatments i-e. 170 ºC and 120 ºC have high flavonoid contents (0.93±0.01 mg/g and 0.70± 0.02 mg/g) as compared to aqueous and methanolic extract of lemongrass. Aqueous extract and methanolic extract flavonoid contents were (0.39 ±0.43 mg/g) (0.29±0.02 mg/g). The capability of different solvents to extract the total flavonoid content (TFC) is given in the order as treatment with temperature in the form of infusion/ decoction > methanol > *n*- hexane > aqueous > ethanol (Atoui *et al.*, 2005; Geetha *et al.*, 2005).

Figure 1 (a) graphically represents the relationship between the phenolic contents found in lemongrass extracts at different temperature treatments (120˚C and 170˚C) compared to aqueous and methanolic extracts. Significant high results were indicated by '**' signs. Figure 1 (b) represents high flavonoid content of methanolic extract of lemongrass compared to aqueous

extract. Similarly, extract of lemongrass at temperature 170 ˚C and 120 ºC treatment have high flavonoid contents as compared to aqueous and methanolic extracts. Flavonoid and tannins seem to be most important promising polyphenol compounds that quenching singlet oxygen and many electronically excited molecules and progression of many degenerative diseases (Sindhu and Kuttan, 2010).

Radical scavenging activity by using DPPH and reducing power assay of different extracts of Lemon grass

Results of DPPH radical scavenging activity and reducing power assay have been given in Table 2. This scavenging activity results indicated that extract of lemongrass at temperature (170ºC and 120ºC) had excessive high antioxidant contents as compared to methanolic and aqueous extracts. DPPH radical scavenging assay results showed that extract at 170ºC temperature causes 83% inhibition of free radicals while extract at 120ºC temperature scavenges 53% of free radicals, as compared to aqueous and methanolic extracts. From the graphical expression, it was found that extract of lemongrass has high value of percentage inhibition of DPPH as compared to other extracts of lemongrass. It was concluded based on results that when the plants are extracted at different temperatures, the boiling must not be very long and the plant samples must not be ground very finely since this leads to loss of components of the plant material in the extracts (Singh *et al.*, 2007). Furthermore, the industrial and drying processes also affect the essential oil content of the plant samples (Barbosa *et al*., 2008). Significant high results were indicated by '**' signs.

Results given in table 2 , it was observed that the extracts of lemongrass had high percentage of reducing power (68%) at temperature 170 ºC treatment while at 120 ºC have high reducing power percentage (60%) as compared to aqueous and methanolic extract of lemongrass. It was observed that the yellow color is changed into bluish by the reduction of ferric ions into ferrous ions. The intensity of the color indicates the low activity of functionality of the compounds which was present in the medium. Greater the color intensity more would be the active function of the compounds and vice versa (Zou *et al.*, 2004). Figure 2 results indicate that (a) , methanol extract have minimum scavenging activity (48 %) at a 100 µg/µL concentration as compared to other tested extracts at the same concentration

Figure 2 (b), results also shows that percentage of reducing power of aqueous and methanolic extract of lemon grass was decreased as compared to other extracts at different temperature treatments. The decreasing capability of compound might also serve as indicator of its potential

antioxidant activity. Bourgou *et al*., (2008) studied that there were antioxidants which reduce the ferric cyanide into ferrous ions. Saeedeh *et al*. (2007) reported that aqueous extracts contain lowest scavenging ability that was comparable with results reported in this paper.

Antimicrobial activity of Lemon grass (*Cymbopogan citratus*)

The lemongrass extracts were subjected for the antimicrobial activity. The results showed that the extracts of lemongrass have broad-spectrum effect toward few microbial species forming clear zone of inhibition. The average values of different extracts of the lemongrass were used for the calculation of zone of inhibition as well as index activity.

Antibacterial assay

All extracts exhibited comparable antimicrobial activity against the bacterial species. From the results shown in Table 3 it was observed that the activity index of methanolic extracts of lemongrass was greater as compared to other extracts. Activity index of methanolic extract against *B.subtilis* was (0.72±0.14) while against *S.aureus* (0.67±0.09) that was high as compared to other extracts against these gram positive bacterial species. In case of *E.coli* methanolic extract has (0.72±0.02) activity index that was almost same as in case of *B.subtilis.* While in case of *P.multocida,* methanolic extract of lemongrass has (0.80±0.07) activity index and (0.78±0.01) was observed against *A.tumefacians* gram negative bacterial species. It was also noted that methanolic extract of lemon grass has high value of activity index against *P.multocida* bacterial strains among other strains. Extracts at different temperature (120 ºC and 170 ºC) of lemongrass has low activity index among all the selected gram positive and gram negative bacterial species. This is due to fact most of the antimicrobial compounds are more soluble in methanol as compared to aqueous medium which showed high activity. Chloramphenicol and autoclaved distilled water were used as positive and negative controls, respectively.

It was also clear from Figure 3(a), low activity index was observed at 120°C and 170°C extract of lemongrass while high activity index was found in the methanolic extracts as compared to other extracts. Similarly, from Figure 3(b), it was shown that methanolic extract of lemongrass have high activity index as compared to other extracts against selected Gram negative bacterial species. While aqueous extract of lemongrass has low activity index against *E. coli* while methanolic extract have high inhibition zone and activity index.

It was observed from the results that the lemongrass extracts at temperature 120°C and 170°C were less active as compared to the aqueous as well as methanolic extracts which have activity

index (0.54±0.01 and 0.51±0.07) at above mentioned temperatures against gram positive (*B. subtilis* and *S. aureus)* bacterial strains and gram negative (*E. coli*, *P. multocida* and *A. tumefacians*) bacterial species. Similar trend in results was observed in Naz and Bano (2012). The reason behind this is that most of the antimicrobial compounds might be less active in water or extracts that was treated at high temperature (170 ºC and 120 ºC) which ultimately indicated decrease activity index in comparison with aqueous and other extracts. The results of the solubility and inhibitory impact of the antimicrobial compounds in methanol was found greater as compared to the ethanol and water. The similar trend was discussed by Naz *et al.* (2011) and Aiyegoro *et al*. (2008). The trend in the activity index results of this paper for various lemongrass extracts was shown by given as; methanolic extract> aqueous extracts> extract at 170 ˚C and 120 ˚C.

Minimum Inhibitory concentration (MIC) and percentage inhibition of biofilm of different extracts of lemon grass

From the results shown in table 4, value of MIC (1.59±0.32 µg/µL, 1.58±0.43 µg/µL and 1.66±0.56 µg/µL) was least value at which the growth of gram negative (*E. coli, P.multocida* and *A.tumefacians*) bacteria occurred against methanolic extract of lemongrass. It was indicated that the least concentration of the sample which does not inhibit the growth of microorganism as compared to other extracts that was comparable with standard antibiotics (1.58±0.34 µg/µL, 1.78±0.10 µg/µL and 1.69±0.08 µg/µL) respectively. Similarly, extract of lemongrass at 170ºC have high value of MIC (1.87±0.40 µg/µL) against *E.coli*. extract of lemongrass at 120ºC having (2.11±0.39) MIC value against *P.multocida* and (2.42±0.45) against *A.ttumefacians* respectively.

Methanolic extract of lemongrass have least value of MIC (1.84±0.60µg/µL) against *B.subtilis* and (1.37±0.57µg/µL) against *S. aureus* of gram positive bacterial species. From the above mentioned antimicrobial test results it was clear that methanolic extract of lemongrass inhibit the growth of bacteria at low concentration against gram positive (*S.aureus)* species as compared to tested gram negative bacterial species. From previous reported work, it was concluded that methanolic solvent was most efficient for the extraction of antimicrobial compounds from the lemongrass. Phytochemical compounds could be more responsible for the antibacterial activity (Ashraf *et al.*, 2005; Bupesh 2007).

From Figure 4, high percentage of biofilm inhibition (71.54±0.23) was observed in methanolic extracts of lemongrass as compared to aqueous extract of lemon grass having percentage

inhibition of biofilm (65.12±0.23). Similarly extract of lemongrass at different temperature treatments (170ºC and 120ºC) have percentage of biofilm inhibition (55.43±0.32 and 45.55±0.43), respectively. Antibiotic that taken as positive control has low value of optical density designate the minimum growth of bacteria due to presence of antibiotic that inhibit the growth of bacteria (80.43±0.23). While negative control has maximum growth of bacteria that leads to the formation of biofilm because no antibiotic present there and bacteria easily grow. Citral, a compound known to possess antimicrobial activity, is monoterpene an aldehyde which could disrupt microbial cell membrane stability and cause membrane leakage (Wang and Sun, 2013).

Conclusion

The results of this research work have found that *Cymbopogon citratus* as a potential good source of therapeutic agents. It was found that the most powerful solvents for the separation of antimicrobial compounds were the methanolic extracts. It was also shown that *Cymbopogon citratus* have strong potential for antioxidant compounds such as phenolics and flavonoids. There was greater contribution in research on cleaning of bioactive compounds, under suitable conditions and provides restraint against microorganisms. Moreover, the study has shown that the extracts of lemon grass treated with different temperature treatments provide the new way of medical justification in the use of medicinal plants.

Conflict of interest

The authors declare no conflict of interest. This paper has not been submitted for publication to any other journal.

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Table 1. TPC and TFC (mg/g) of different extracts of lemongrass

Sr. No	Sample extracts	TPC (mg/g)	TFC mg/g
		Mean ±SD	Mean ±SD
1	Aqueous extract of lemongrass	0.37±0.03	0.39±0.43
2	Methanolic extract of lemongrass	0.23±0.02	0.93±0.02
3	Heat-treated extract of lemongrass at 170°C temperature	1.25±0.10	0.70±0.01
4	Heat-treated extract of lemongrass at 120°C temperature	1.08±0.24	0.29±0.02

Table 2: IC_{50} and reducing power of different extracts lemongrass

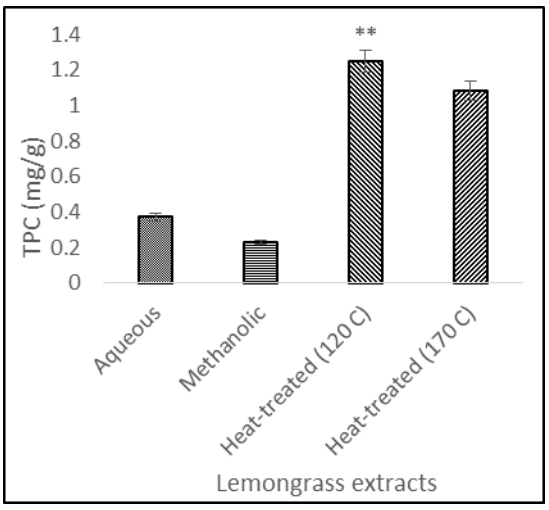
	Sample extracts	IC_{50}	Reducing power
		Mean ±SD	Mean ±SD
1	Aqueous extract of lemongrass	34±0.11	23 ± 0.07
2	Methanolic extract of lemongrass	48±0.27	21 ± 0.11
3	Extract of lemon grass at 170°C temperature	83±0.04	68±0.01
4	Extract of lemon grass at 120°C temperature	53±0.15	60±0.16

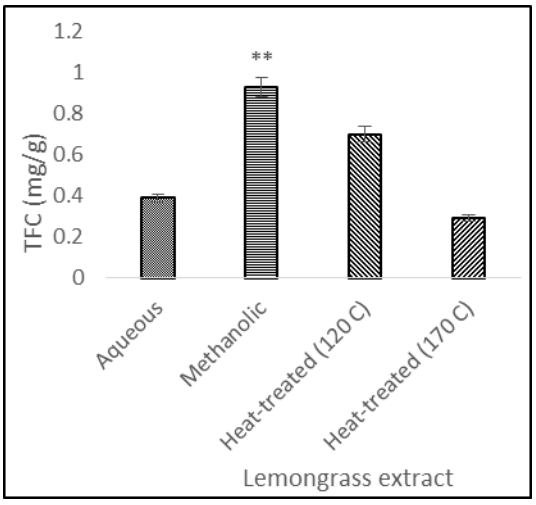
Table 3. Activity index and zone of inhibition (mm) of different extracts of Lemongrass against Gram positive and Gram negative bacterial strains.

Sr. No	Sample extracts	Gram positive bacteria				Gram negative bacteria					
		B. subtilis		*S. aureus*		*E. coli*		*P. multicoida*		*A.tumefacians*	
		Zone of inhibition (mm)	Activity index	Zone of inhibition (mm)	Activity index	Zone of inhibition (mm)	Activity index	Zone of inhibition (mm)	Activity index	Zone of inhibition (mm)	Activity index
1	Aqueous extract of lemongrass	11±1.04	0.54±0.09	13±0.76	0.60±0.04	15.18±1.07	0.70±0.01	14±2.00	0.61±0.09	11.33±0.76	0.52±0.07
2	Methanolic extract of lemongrass	10.33±1.53	0.51±0.07	11±1.04	0.51±0.07	13.17±1.04	0.60±0.09	10.7±1.15	0.47±0.06	11.83±0.76	0.55±0.07
3	Heat-treated lemongrass extract at 170˚C	12.8±1.04	0.65±0.05	11±0.90	0.59±0.06	13±1.04	0.72±0.02	12±1.04	0.80±0.07	13±0.76	0.78±0.01
4	Heat-treated lemongrass extract at 120˚C	14±2.02	0.72±0.14	12.8±1.04	0.67±0.09	12.5±1.32	0.69±0.15	11.5±0.5	0.72±0.12	12.5±1.32	0.74±0.02
5	Rifampicin (positive control)	22±1.75	1±0.00	22±1.75	1±0.00	23±1.73	1.00±0.00	22.6±1.15	1.00±0.00	21.6±1.52	1.00±0.00

Table 4. Minimum inhibitory concentration (MIC) of different extracts of lemongrass against Gram positive bacteria and Gram-negative bacteria

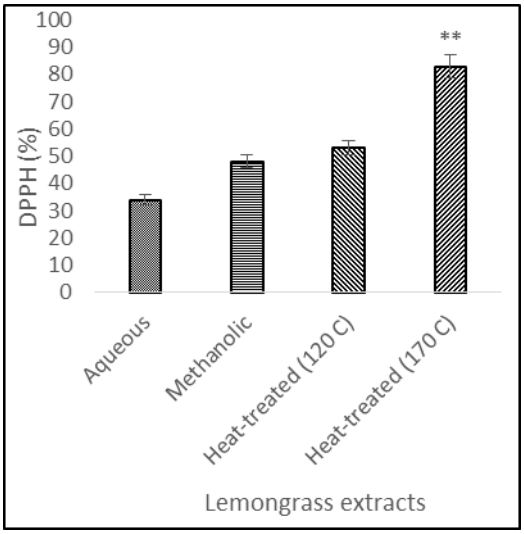
Sample extracts	Minimum Inhibitory Concentration (MIC) µg/µL				
	Gram positive bacteria		Gram negative bacteria		
	B.subtilis	*S. aureus*	*E. coli*	*P.multicoida*	*A.tumefaciens*
Auqeous extract of lemongrass	2.45±0.51	1.52 ± 0.54	1.62±0.53	1.99±0.37	2.20±0.44
Methanolic extract of lemongrass	2.41±0.60	2.20 ± 0.57	1.87±0.32	2.11±0.43	2.42±0.56
Heat-treated extract of lemongrass at 170^0C	1.92±0.15	1.37± 0.34	1.59 ±0.40	1.67 ±0.26	1.73 ±0.41
Heat-treated extract of lemongrass at 120^0C	1.84±0.19	1.48 ± 0.15	1.64 ±0.30	1.58 ±0.39	1.66 ±0.45
Positive control (Rifampicin)	1.86±0.06	1.91 ± 0.35	1.58±0.34	1.78±0.10	1.69±0.08
Negative control	1.80±0.04	1.35 ±0.23	1.86±0.10	1.86±0.12	1.78±0.06

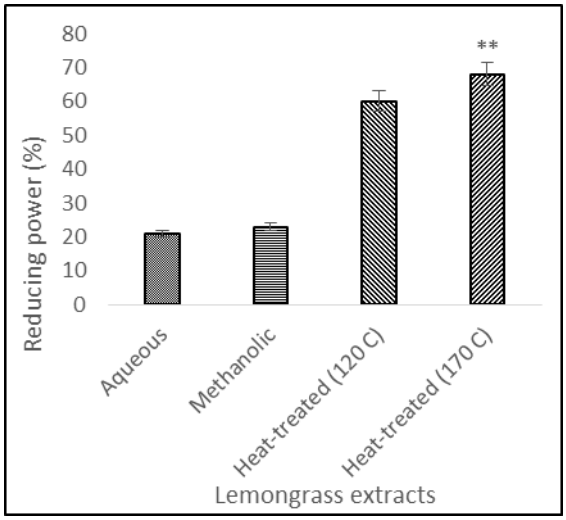




(a) **(b)**

Figure 1. (a) Total phenolic contents and (b) Total flavonoid contents of different extracts of lemongrass





(a) **(b)**

Figure 2: Percentage of DPPH assay and percentage of reducing power of different extracts of lemongrass

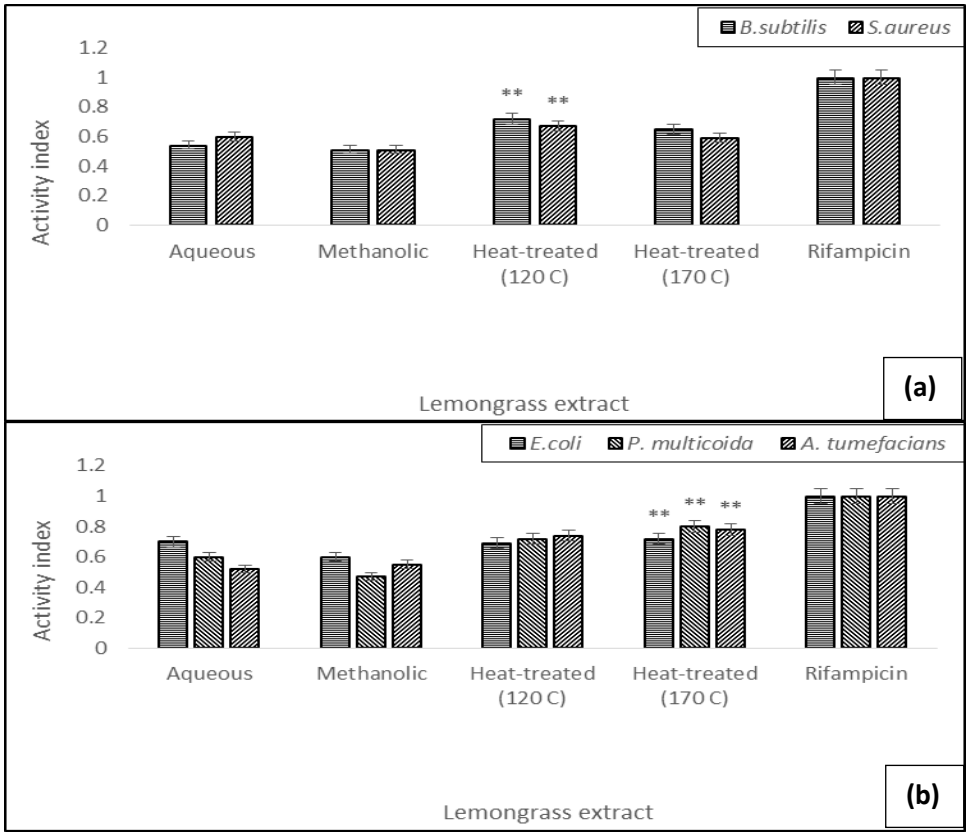


Figure 3. Antibacterial activity of different extracts against (a) Gram positive and (b) Gram negative bacterial strains

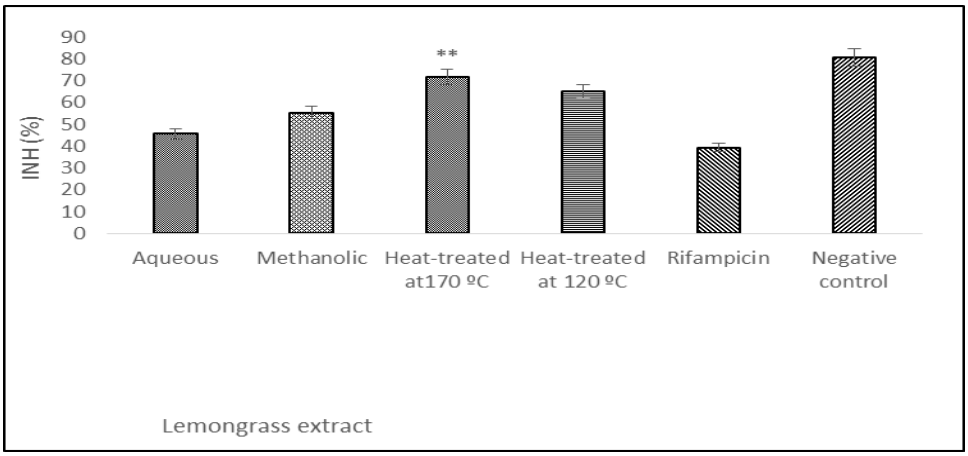


Figure 4. Percentage inhibition of biofilm through different extracts of lemon grass