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Antibacterial Activity of Lemongrass on Gram Positive and Gram Negative Bacteria of Human Pathogens

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Abstract

All leaves were thoroughly washed with sterile distilled water and dried in shade until all moisture evaporated. About 200 gram of leaves were grinded with 500 ml water for aqueous juices extraction for triplicate times. Similarly, essential oil was isolated triplicate times from leaves by 3 hour hydrodistillation of 200 gram of leaves in 500 ml of water. The essential oil and aqueous juices extraction was separated and residues obtained were mixed with equal volume of methanol for obtaining methanol extraction of juices and oil. Then, juices extraction and oil extraction were mixed diluted to 0.5%, 1%, 2.5%, 5%, and 10% with DMSO. Agar well diffusion method was done for studied antimicrobial activity for the pure bacterial cultures of *Escherichia coli, Staphylococcus aureus*, *klebsialla pneumonia*, *Salmonella* Typhi, *Pseudomonas aurogenosa* and for quality control *Staphylococcus aureus* (ATCC: 25923) and *Escherichia coli* (ATCC: 25922). A cork borer of 7 mm diameter selected for 70µl pouring in each well of different concentration along positive control (oflaxacin) and negative control (Dimethyl sulfoxide) then incubated at 37°C in an incubator for 24 hrs to 48 hrs in aerobic condition. After incubation, clear zone of inhibition were observed around the wells. Oil extraction of lemon grass was found to be highly effective for gram positive bacteria *S. Aureus* (up to 42 mm- Methanol) and gram negative bacteria *S. Typhi* (upto 36 mm methanol). It was concluded that oil extraction of leaves of lemongrass were found to be highly effective for treatment of human pathogens.

Keywords: Antimicrobial activity,*Cymbopogon citratus*, Extraction, Well diffusion method

Introduction

Plant essential oils and extracts have been used for many thousands of years, in food preservation, pharmaceuticals, alternative medicine and natural therapies. It is continuously used as traditional treatment (Burt, 2004). Among different medicinal plants, *Cymbopogon citratus* is one which is commonly known as Lemongrass. *Cymbopogon citratus* (Lemongrass) belongs to Poaceae family which is a perennial plant with long, thin leaves one of the largely cultivated medicinal plants for its essential oils in parts of tropical and subtropical areas of Asia, Africa and America (Chanthal et al., 2012). Lemon is regarded as one of the grass which is commonly available in Nepal and abroad.

It is widely used in different conditions of pain and discomfort. The oil obtained from the grass has diverse medicinal value. It also produces semi-synthetic Vitamin A that reduces the risk of Xerophthalmia and Night blindness. The grass has great benefits to mankind as it revitalizes the body and mind, helps with infections and act as muscle and skin toner (Srivastava et al., 2013). The leaves of lemongrass present lemony characteristic flavor due to its main content, citral which present great importance to the industry. There were a number of studies carried out to prove the anti-oxidant, anti-microbial and anti-fungal activities of lemongrass (Nikos & Costas, 2007; Oloyede et al., 2010). It is commonly used in folk medicine for treatment of nervous and gastrointestinal disturbances. However, it is also used as antispasmodic, analgesic, anti-inflammatory, anti-pyretic, diuretic and sedative (Dubey et al., 2011).

Essential oils are a concentrated hydrophobic liquids containing volatile chemical compounds from plants materials and do not only originate from flowers, but from herbs, trees and various other plant material. It is estimated that the global number of plants is of the order of 300,000 and about 10% of these contains essential oils and could be used as a source for their

production (Husnu & Gerhard, 2010). The common methods to extract essential oil from medicinal plant, including for lemongrass *(Cymbopogon citratus)*, are hydrodistillation (HD), steam distillation, steam and water distillation, maceration, empyreumatic (or destructive) distillation and expression (Ashgari et al., 2010).

Lemongrass has phytoconstituents such as tannins, flavanoids, alkaloids and various essential oils in this herb. Secondary active metabolites of a number of components have also been implicated in the varied pharmacological effects of this plant. Lemongrass possesses various antimicrobial properties. The extracts of lemongrass leaves (dried) with cold, hot and different solvents like ethanol and methanol were screened for its antimicrobial activity against various bacteria like *Bacillus vallismortis, Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Vibrio cholerae*. Lemongrass extracts possess a great antimicrobial activity against the antibiotic resistant microorganisms (Isam et al., 2009).

Due to the development of resistance in pathogenic microorganisms to antibiotics used in modern medical science, there is a growing attention towards plant extracts as a source of new antimicrobial drug discoveries. As such investigations on the composition, activity, as well as validation of the use of extracts obtained from medicinal plant is important. The emergence of drug resistance in human and animal pathogenic bacteria, as well as undesirable side effects of certain antibiotics, has triggered immense interest in the search for new antimicrobial alternatives of plant origin. The purpose of this study is to identify the antibacterial activity of the different extract of leaves against the growth of bacteria.

Materials and Methods

Plant material collection

For the study, only leaf extract was used. So, the leaves of the mature plants of lemongrass were collected aseptically from ground of Tribhuvan University, Kirtipur, in clean plastic bag and transported to Laboratory. Then further extraction procedure followed according to Ezekwesili et al. (2004) and Mishra & Mishra (2011).

Preparation of leaf extract

All leaves were thoroughly washed with tap water and then rinsed with sterile distilled water. Then all leaves were dried in shade until all moisture evaporated.

Aqueous extraction of leaves (Juices extraction)

About 200 grams of leaves were ground with 500 ml water and kept 8 hrs at ambient temperature. Then whole mixture was filtered using a cheese cloth and obtained extracts was centrifuged. After centrifugation, supernatant was labeled it and used for antibacterial activity assay. Residue obtained from filtration was used for methanol extraction.

Extraction of essential oil

Essential oil was isolated from leaves by 3 hrs hydrodistillation of 200 grams of leaves which were placed in 500 ml of water. The essential oil was separated, dried over anhydrous sodium sulphate and stored at -20°C until used for other tests.

Alcoholic extraction

Both types of residues obtained after aqueous extraction of juices and essential oil extraction both were also separately treated with equal volume of methanol and kept for 24 hrs at ambient conditions. Then the mixtures were filtered using cheese cloth and extracts were obtained. In half pure extraction (crude) of lemongrass of juices and oil, no dilution were done and also used for study antimicrobial activity.

Dilution of juices and oil extraction

The juices extraction and oil extraction were diluted to 0.5%, 1%, 2.5%, 5%, and 10% with dimethyl sulphoxide (DMSO). Similarly, methanol extraction were diluted by DMSO as similar percentage.

Preparation of microbial cultures

Pure bacterial cultures were used for the study and maintained on nutrient agar medium by subculturing of *Escherichia coli*, *Staphylococcus* species, *Klebsialla pneumonia*, *Salmonella typhi* and *Pseudomonas aurogenosa*. ATCC culture of bacteria with *Staphylococcus aureus* (ATCC: 25923) and *Escherichia coli* (ATCC: 25922) were used for quality control for study of antibacterial activity of bacteria. All bacteria were obtained from Med-Micro Research Laboratory, Kathmandu, Nepal.

Antimicrobial activity

Agar well diffusion method was used to determine the antimicrobial activity of leaves extract in vitro. This method was done triplet times and average mean was taken as accurate mean for zone of inhibition of plants extracts. The pure colonies (3-4 colonies) of bacteria were transferred to nutrient broth and incubated for 4 hrs. then their turbidity was compare with 0.5 McFarland turbidity standards. After adjusting the inoculums, a sterile cotton swab was dipped into the inoculums and rotated against the wall of the tube above the liquid to remove excess inoculums. Then the swab was lawn culture on MHA plate by the entire surface rotated by approximately 60º between streaks to ensure even distribution.

The inoculated plate was allowed to stand for at least 3 minutes but no longer than 15 min, before making wells for different compounds to be tested. A hollow tube or cork borer of 7 mm diameter was sterile and press above the inoculated agar plates. It was removed immediately by making a well in the plate; likewise, other wells on each plate were made, one each for positive control (oflaxacin), negative control (DMSO) and for five respective concentrations (0.5%, 1%, 2.5%, 5%, and 10%).

Micropipettes were used to place 70µl of the solution of respective dilution in each well. Plates were incubated at 37ºC in an incubator for 24 hrs to 48 hrs in aerobic condition. After incubation clear zone of inhibition were observed around the wells. Diameters of those inhibition zones were measured and compare it with ATCC bacteria with *Staphylococcus aureus* (ATCC: 25923) and *Escherichia coli* (ATCC: 25922).

Results and Discussion

In this study, the antibacterial activity of lemongrass extracts was determined against five human pathogenic bacteria by the well diffusion method. Aqueous (juices) and methanol extracts of leaves were test for antibacterial activity. The different human pathogenic bacteria (5) were *S. aureus, E. coli, K. pneumoniae, S.* Typhi and *P. aeruginosa*. On the other hand, ATCC culture of *E. coli* (ATCC: 25922) and *S. aureus* (ATCC: 25923) were used for quality control. The well containing ofloxacin antibiotic was positive control which showed inhibiting activity for all the five bacteria. On the other hand, the blank well containing DMSO as negative control which did not show any inhibiting activity for all bacteria included for antibacterial activity.

From the present study it is clear that lemongrass juices and oil possess anantibacterial activity against gram positive and gram negative bacteria. In juices extraction, direct lemon grass juices of low dilution (0.5%) only showed antibacterial activity for ATCC gram positive bacteria *S. aureus* (ATCC: 25923). The effective of lower dilution of extraction is due to easily diffusion of lower concentration of extraction in media for antibacterial activity. On the other hand, methanol extraction of lemon grass juices of all dilution showed antibacterial activity for ATCC gram positive bacteria *S. aureus* (ATCC: 25923). However, result obtained by Nyamath & Karthikeyan (2018) in which cold water extraction (juice) of lemongrass was found to be effective than hot water, methanol and ethanol extraction which result is interesting in the traditional method of treating a bacterial infection.The results obtained two of these methods differ due to many factors between assays (Janssen et al., 1987; Hili et al., 1997). However, direct lemongrass juices and methanol juices extraction did not show antibacterial activity for human pathogenic bacteria. The results obtained support the general indication that gram positive

organisms are more sensitive to lemon grass juices than gram negative bacteria.

The effective antibacterial activity was found in oil extraction of lemongrass leaves than juices extraction. Among different dilution of oil extraction of lemongrass leaves, both pure extraction and all dilution of extraction showed the highest zone of inhibition on gram positive than gram negative bacteria. So, gram positive bacteria were found to be more sensitive to the oil extraction than gram negative bacteria except *P. aeruginosa* and *K. Pneumoniae*. Similar observations were made by Onawunmi & Ongulana (1986) and Cimanga et al., (2002), *P. aeruginosa* and *K. Pneumoniae* were found resistant at all the concentration of lemongrass oil including pure (crude). Similar results were reported by Onawunmi et al. (1984), Alam et al. (1994), Marta et al. (2004), Pereira et al. (2004) and Naik et al. (2010), *P. aeruginosa* was found to be resistant to all dilution of oil extraction of lemongrass. This might be due to the multidrug resistant *P. aeruginosa* and *K. Pneumoniae* which was found to be non-effective with juices and oil extracts of all dilution of lemongrass.

Among two methods of extraction, methanol extraction was found to be more effective than aqueous oil extraction of lemon grass. The highest zone of inhibition was observed in *S. aureus* (ATCC: 25923) and *S. aureus* from methanol oil extraction than aqueous oil extraction of lemongrass. Among gram negative bacteria, antibiotic resistant bacteria like *S.* Typhi, showed highest antibacterial activity than *E. coli.* The results obtained by each of these methods differ due to many like microbial growth, exposure of microorganisms to the oil, the solubility of oil or oil components and the use and quality of an emulsifier etc. mentioned by Naik et al. (2010).

According to Isam et al. (2009) showed lemongrass extracts possess a great antimicrobial activity against the antibiotic resistant microorganisms. Lovet et al. (2010) mentioned that the presence of phytochemical and bioactive ingredients in lemon grass have been attributed to its antimicrobial potentials. So, it is widely used in different conditions of pain and discomfort (Srivastava et al., 2013). The lemongrass oil obtained from the grass has diverse medicinal value. The leaves are used in the treatment of cough, fever, depression, nervous disorder and skin irritations. So, essential oil is one of the important components of lemongrass extracts and its applications will be helpful in treating different infections.

Table 1: Antibacterial activity of juices of aqueous and methanol extraction of lemongrass leaves

Extraction	Juices extract zone of inhibition (mm) with different dilution						
	Bacteria	**0.5%**	**1%**	**2.5%**	**5%**	**10%**	**Pure juices**
Aqueous extraction	*S. aureus*(ATCC: 25923)	13	0	0	0	0	0
	S. aureus	0	0	0	0	0	0
	E. coli (ATCC: 25922)	0	0	0	0	0	0
	E. coli	0	0	0	0	0	0
	K. pneumoniae	0	0	0	0	0	0
	S. Typhi	0	0	0	0	0	0
	P. aeroginosa	0	0	0	0	0	0
Methanol extraction	*S. aureus*(ATCC: 25923)	8	9	11	12	14	0
	S. aureus	0	0	0	0	0	0
	E. coli (ATCC: 25922)	0	0	0	0	0	0
	E. coli	0	0	0	0	0	0
	K. pneumoniae	0	0	0	0	0	0
	S. Typhi	0	0	0	0	0	0
	P. aeroginosa	0	0	0	0	0	0

Table 2: Antibacterial activity of oil of aqueous and methanol extraction of lemongrass leaves

Extraction	Bacteria	Essential oil extracts zone of inhibition (mm) with different dilution 0.5%	1%	2.5%	5%	10%	Pure oil
Aqueous extraction	*S. aureus*(ATCC: 25923)	8	10	28	30	36	20
	S. aureus	10	15	20	35	42	30
	E. coli (ATCC: 25922)	0	0	0	0	0	0
	E. coli	0	0	0	0	0	0
	K. pneumoniae	0	0	0	0	0	0
	S. Typhi	12	15	20	36	38	24
	P. aeroginosa	0	0	0	0	0	0
Methanol extraction	*S. aureus*(ATCC: 25923)	30	32	30	40	42	30
	S. aureus	20	24	26	36	40	25
	E. coli (ATCC: 25922)	8	8	10	10	11	0
	E. coli	8	0	0	0	0	0
	K. pneumoniae	0	0	0	0	0	0
	S. Typhi	18	20	25	30	36	38
	P. aeroginosa	0	0	0	0	0	0

Plate 1: Hydrodistillation of 200 gram of lemongrass leaves in 500 ml of water

Plate 2: Extraction of oil of lemongrass

Plate3: Antimicrobial activity of *Staplylococcus aureus* (ATCC: 25923) on lemongrass with pure (crude), 10%, 5%, 2.5%, 1% and 0.5% dilution

Conclusion

From this research work, it can be concluded that oil extraction of Lemongrass was found to be highly effective for gram positive bacteria *S. aureus* and gram negative bacteria *S.* Typhi. So, oil extraction of lemongrass showed antibacterial activity for human pathogens and suggested that use of Lemongrass oil would be helpful in the treatment of infections caused by different bacteria.

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