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RESEARCH ARTICLE

STUDY ON DISINFECTANT POTENTIAL OF LEMON GRASS OIL AGAINST COMMON PATHOGENS.

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Abstract

Essential oils of aromatic plants species are used in industries for the production of soaps, perfumes and toiletries. Many of them are also used in traditional medicine for various purposes. Investigations concerning the evaluation of the biological activities of essential oils of some medicinal plants have revealed that some of them exhibited antibacterial, antifungal and insecticidal properties. Lemon grass oil, extracted from the herb *Cymbopogon citratus* is one of the essential oils known for its various properties. The present study focused on the laboratory investigation of the effectiveness of lemon grass oil as a disinfectant and compares its activity with a commonly used chemical disinfectant. Surface disinfection test was performed to assess the effectiveness of the three disinfectants under the conditions in which they would be used. The result of present study suggests the use of lemon grass oil as a safe natural alternative to chemical disinfectants. It is effective in controlling pathogenic microorganisms and can be used in undiluted form for disinfecting highly contaminated areas while only 1:2 or 1:4 dilutions is needed in areas of low pathogenic density. As a natural plant derived volatile oil, it is safe, non-toxic and environment friendly when compared with other chemical disinfectants.

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Introduction:-

Disinfectants are agents that destroy or inhibit the growth of pathogenic micro-organisms to a safer level. Disinfectants are applied to inanimate objects and materials such as instruments and surfaces to control and prevent infection while antiseptics are types of disinfectant that destroy or inhibit growth of microorganisms on living tissues without causing injurious effects when applied to surfaces (Oterzio et al., 1961). Antiseptics and disinfectants are extensively used in the hospitals, houses or public places on a variety of topical and hard surface applications. They are an essential part of infection control practices and measures that ensure clean and hygienic environment (Barker et al., 2003). Mounted concerns over the potentials for microbial contaminations and infections risks in the food and general consumer markets have also led to increased use of antiseptics and disinfectants by the general public.

A wide variety of active chemical agents (or "biocides") have been used as antiseptics and disinfectants for hundreds of years (Schaffer et al., 1930). The common chemical disinfectants include phenolics, chlorhexidine, chlorine compounds, alcohols, iodine, formaldehyde, glutaraldehyde etc. The widespread use of chemical disinfectants causes serious environmental problems in addition to the development of microbial resistance against antiseptics and disinfectants. Natural disinfectants can be developed as an alternate choice against the problems caused by the use of chemical disinfectants.

Essential oils of aromatic plants species are used in industries for the production of soaps, perfumes and toiletries. Many of them are also used in traditional medicine for various purposes. Investigations concerning the evaluation of the biological activities of essential oils of some medicinal plants have revealed that some of them exhibited antibacterial,

antifungal and insecticidal properties (Burt,2004) Lemon grass oil, extracted from the herb *Cymbopogon citratus* is one of the essential oils known for its various properties. The tea made from its leaves is popularly used in Brazil as antispasmodic, analgesic, anti inflammatory, antipyretic, diuretic and sedative (Carlini et al., 1986) The volatile oil obtained from fresh leaves of this plant is widely used by the perfumes and cosmetics industries. It is a well known analgesic and anti-inflammatory agent which has also been used as an antidepressant. The lemon grass oil is also reported to have antipyretic, astringent, deodorant, diuretic properties. Lemon grass oil is characterized for monoterpene compounds, and citral is found to be the major component present at the level of approximately 65-85%. In addition to citral, the lemongrass oil consists of small quantities of geraniol, geranylacetate and monoterpene olefins, such as myrcene (Ferreira et al., 1989). The study focused on the laboratory investigation of the effectiveness of lemon grass oil as a disinfectant and compares its activity with a commonly used chemical disinfectant.

Materials and methods:-

Pure cultures of *Escherichia coli* and *Staphylococcus aureus* maintained in the laboratory was used for the study. The Lemon Grass Oil was purchased from the local market and stored in the laboratory for the study. The chemical disinfectant, Lysol used in the study was purchased from the local market.

Preparation of Agar Plates and Cotton Swabs:-

Nutrient agar was prepared as per the required composition, aseptically poured into the petri dishes and solidified. The solidified nutrient agar plates were stored for further use. Nutrient agar broths were also prepared in test tubes. All the media were stored at 4°C. Cotton swabs were prepared and sterilized by autoclaving and stored in the refrigerator.

Preparation of Lemon grass oil dilutions:-

Different dilutions of lemon grass oil were disinfectants were made with sterile water just before the experiment. The dilutions of 1:1, 1:2, 1:4 along with undiluted lemon grass oil were used for assessing the disinfectant potential.

Preparation standard disinfectant solution:-

The chemical disinfectant Lysol was used as the standard control of the experiment. The Lysol was diluted as per the instruction by the manufacturer (12 ml of Lysol in 4 litres of water). The diluted as well as undiluted Lysol solution was used as the standard disinfectant for the study.

Surface Disinfection Test:-

Surface disinfection test was performed to assess the effectiveness of the three disinfectants under the conditions in which they would be used. An area of 50 square inches was marked on the floor for testing each sample and also for control of the experiment. The selected area was cleaned thoroughly and wiped with 75% alcohol 72 hrs prior to the test. Different concentrations of lemon grass oil and standard disinfectant solution was prepared. The sterilized cotton swabs were dipped in the test solutions and the test floor area was thoroughly wiped with cotton swabs. Smears were taken at regular time intervals with a sterilized cotton swab and inoculated into nutrient agar plates. All the plates were incubated at 37°C overnight and the grown colonies were counted.

Disc diffusion Method:-

Petriplates and paper discs were sterilized and kept for the test. Nutrient agar medium was prepared and sterilized by autoclaving. Inside the Laminar air flow chamber, 1 mL of bacterial culture was aseptically transferred into the sterile petriplate. The molten nutrient agar at bearable temperature was added into the petriplate. The agar plates were allowed to solidify. Paper discs were placed on the surface of solidified nutrient agar medium. 0.1 mL of each test dilutions of lemon grass oil and Lysol were applied to the paper disc. The plates were incubated at 37°C overnight. The clearance zone around the paper discs was measured.

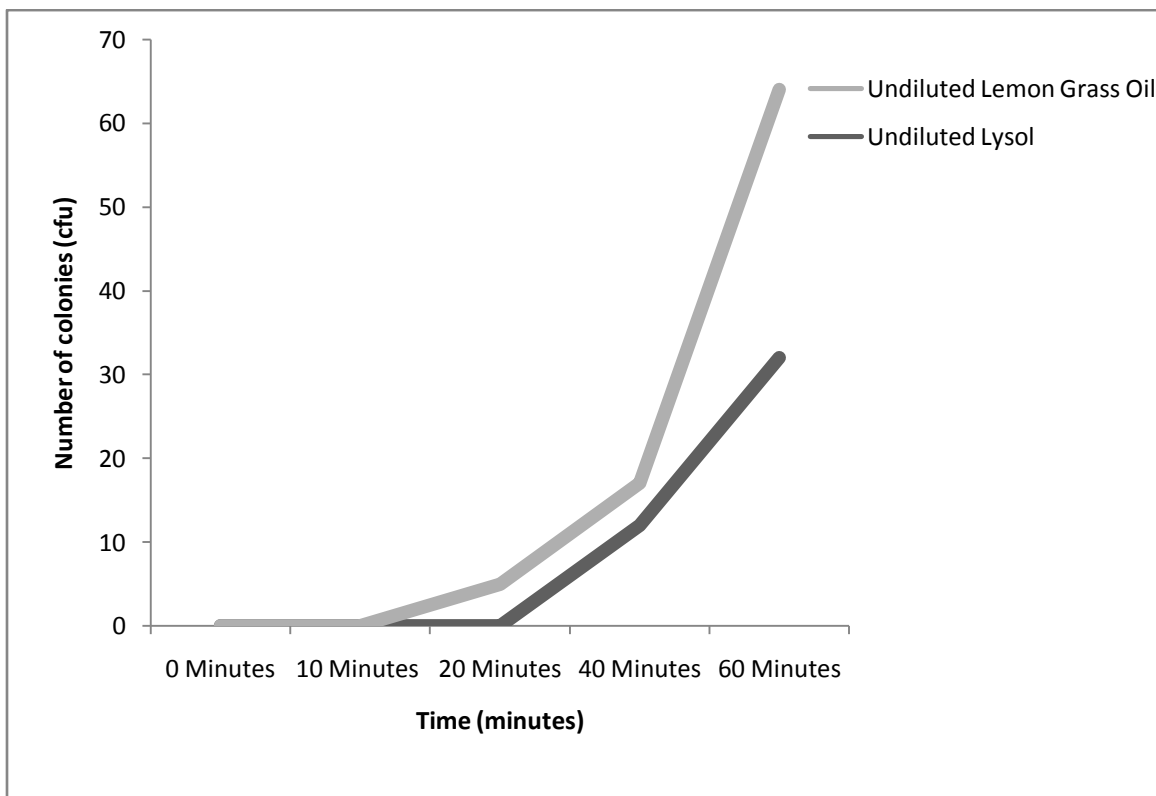
Result:-

Surface disinfection test:-

Surface disinfection test of the standard chemical disinfectant Lysol in undiluted as well as in dilution were showed to be effective in controlling microbial population for the test period of 60 minutes (Table-1, Figure -1).

Table 1:- Surface Disinfection Test – Lysol.

Test solution		Colony Count after the disinfection				
		0 minutes	10 minutes	20 minutes	40 minutes	60 minutes
Lysol	Undiluted	0	0	0	12	32
	Diluted	0	0	13	24	42
Sterile water (control)		Uncountable	Uncountable	Uncountable	Uncountable	Uncountable

**Figure 1:-** Surface Disinfection Test.

The lemon grass oil was found especially effective as a disinfectant in low dilutions. It was found to be maintaining the effectiveness up to the 1:4 dilutions (Table- 2, Figure -2) The lemon grass oil could limit the bacterial population under safe levels for the 60 minutes test duration.

Table 2:- Surface Disinfection Test – Lemon grass oil.

Test solution		Colony Count after the disinfection (cfu)				
		0 minutes	10 minutes	20 minutes	40 minutes	60 minutes
Lemon grass oil	Undiluted	0	0	5	5	32
	1:1 Dilution	0	0	7	10	46
	1:2 Dilution	0	5	15	24	120
	1:4 Dilution	2	12	180	268	Uncountable
Sterile water (control)		Uncountable	Uncountable	Uncountable	Uncountable	Uncountable

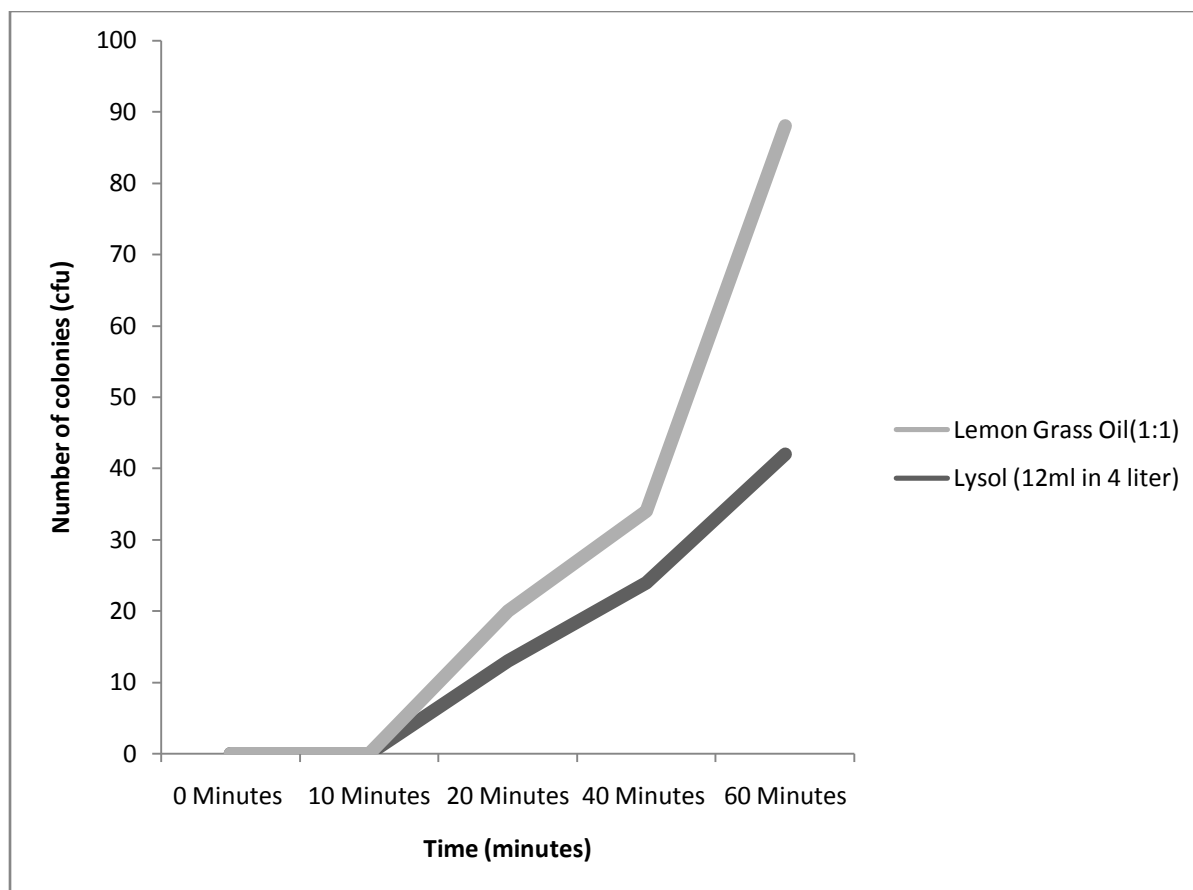


Figure 2:- Surface Disinfection Test

Disc diffusion Method:-

The clearance zones produced by different dilutions of lemon grass oil against *Escherichia coli* and *Staphylococcus aureus* were compared with the zones produced by the chemical disinfectant, Lysol (Table-3). The lemon grass oil produced clear zones of clearance against the two test bacteria in undiluted and 1:2 dilution .which indicates its inhibitory activity against the test organisms.

Table 3:- Disc Diffusion Method.

Test solution	Concentration of test solution	Zone of inhibition in cms	
		<i>S.aureus</i>	<i>E.coli</i>
Lysol	Undiluted	6.4	5.2
	Diluted	4.6	3.8
Lemon grass oil	Undiluted	4.2	3.6
	1:1	2.6	2.1
	1:2	1.4	1.2
	1:4	0.8	0.6

Discussion:-

The primary purpose of disinfection is to control the growth of pathogenic bacteria. Disinfectants are widely used in hospitals, dairies, bottling plants, canneries and other food processing industries. Disinfection is a process during which pathogenic micro – organisms are removed or inactivated by chemical or physical means and an ideal disinfectant should have a broad spectrum of antimicrobial and should be non toxic and non irritating. In the present study the disinfectant potential of lemon grass oil was evaluated. The antibacterial activity and anti fungal activities of lemon grass oil have been reported in previous studies (Burt,2004; Ferreira et al., 1989). It showed very good effectiveness and broad spectrum of activity against *Candida* species (Cristiane et al., 2008).

The lemon grass oil was found to be effective as a disinfectant when compared with the standard chemical disinfectant, Lysol. The percentage of disinfection by the lemon grass oil, when compared to the standard was reasonably high so that it can effectively be used as a disinfectant. The lemon grass oil was highly effective upto 1:2 dilutions and the activity decreased with dilution. The Lemon grass oil was found to control the bacterial population for the test duration of 60 minutes. The disc diffusion method again showed that the lemon grass oil inhibits the growth of bacteria. It produced good zone of clearance. So, lemon grass oil can be used as an effective natural disinfectant instead of chemical disinfectants.

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