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In vitro Antioxidant Potential of an Essential Oils of Lemon Grass, Turmeric Oil and Palmarosa and its Antimicrobial Activity

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Abstract

The purpose of this study was to assess the antioxidant capacity of lemongrass, turmeric and palmarosa essential oils. Through the technique of steam distillation, the essential oil extract was derived from natural sources. Several investigations have shown that essential oils' antioxidant potential is derived from their constituent parts. Here, the antioxidant capacity of the essential oils was assessed using two assays: The Ferric reducing antioxidant power (FRAP) assay and the α, α-diphenyl-β-picrylhydrazyl (DPHH) radical scavenging assay. According to the current study's findings, these plants' essential oils and their individual components can be utilized as natural antioxidants. Compared to lemongrass oil, the essential oils from palmarosa and turmeric have a higher potential for antioxidants. By using FTIR spectroscopy, the materials were characterised in order to evaluate the vibrational groups that were present in them. Aspergillus Niger, Salmonella Typhi, and Escherichia coli were the microbes against which the antimicrobial activity of essential oils was tested.

Keywords: essential oil; antimicrobial activity; antioxidant; DPHH assay; FRAP assay; turmeric oil

1. Introduction:

People have employed spices for medicinal, culinary, and cosmetic purposes since ancient times. The development of patent pharmaceuticals and modernism have led to a decline in the use of natural cures and elixirs. Artificial additives, synthetic medications, and their possible side effects are endangering human life safety. Consequently, there is an increasing trend towards the use of organic materials in cosmetic, pharmaceutical, and culinary applications. Spices refer to aromatic plants and their parts, whether whole or ground, frozen or new, that are primarily used to provide flavor and aroma to food and drinks. Cooking herbs are also regarded as spices in a wider sense. Spices are a vital component in the creation of recipes that reflect the history, society, and geology of a

country. Spices are a vital culinary arts tool when it comes to developing dishes that evoke the history, culture, and geography of a country. Spices, oils, and oleoresins are used in the manufacturing of food and beverages, cosmetics and fragrances, and medications. The commercial production of aromatic herbs for crop protection and food preservation is gaining traction due to the antibacterial and antioxidant properties of numerous spices and their derivatives. With customers displaying a growing antipathy to artificial flavorings and a greater preference for natural and organic products, the future of spices seems bright. Herbs and spices, which are essentially dried leaves, flowers, buds, fruits, seeds, bark, or rhizomes of many plants, are used in cuisine in very little amounts, but since they contain both volatile and fixed oils, they greatly influence flavor and scent.

Anggraeni et al. 2018 reported the free radical induced oxidative stress that influences the occurrence of various degenerative diseases such as cancer, coronary heart disease and premature aging. In the case that body's antioxidant defense system does not have excessive antioxidants, additional natural antioxidant via food or other nutrients intake is needed. Stems of lemongrass *Cymbopogon citratus* Stapf are known to contain phenolic compounds that are known to have antioxidant activity. A study has been carried out to determine antioxidant potential of stems of lemongrass. In this the primary study is to examine essential oil *Cymbopogon citratus* Stapf from Cileles Jatinangor as an antioxidant agent. Essential oil of *Cymbopogon citratus* Stapf was isolated from 1272 g of dried stem by using Karlsruhe steam distillation methods with 0.24% in yield. The product of essential oil was also tested against antioxidant activity DPPH and resulted low activity compare to ascorbic acid and lemongrass oil standard as reference material.

Majewska et al. 2019 reviewed the Lemongrass essential oil comes from the lemongrass plant (Cymbopogon citratus), which grows mainly in tropical and subtropical parts of the world. The chemical constituents of the essential oil which have constantly been detected and determine its biological activity are aldehydes, hydrocarbon terpenes, alcohols, ketones, and esters. The lemongrass essential oil shows a wide spectrum of biological activities. High antibacterial and remarkable antifungal activities make the lemongrass oil a potential food preservative. Mohammad et al. 2021 discussed the prominent cultivation of lemongrass (*Cymbopogon* spp.) relies on the pharmacological incentives of its essential oil. Lemongrass essential oil carries a significant amount of numerous bioactive compounds, such as citral (mixture of geranial and neral), isoneral, isogeranial, geraniol, geranyl acetate, citronellal, citronellol, germacrene-D, and elemol, in addition to other bioactive compounds. These components confer various pharmacological actions including antifungal, antibacterial, antiviral, anticancer, and antioxidant properties. Fatima et al. 2022 stated the food industry is growing vastly, with an increasing number of food products and the demand of consumers to have safe and pathogen-free food with an extended shelf life for consumption. It is critical to have food safe from pathogenic bacteria, fungi, and unpleasant odors or tastes so that the food may not cause any health risks to consumers. Lemongrass is one such natural preservative that possesses significant antimicrobial and antioxidant activity. The essential oil of lemongrass contains a series of terpenes that are responsible for these activities.

Mohsen et al. 2018 stated the Citronella oil was extracted from *Cymbopogon nardus* by ohmic-assisted hydrodistillation (OAHD) and hydrodistillation (HD) and its specifications were studied by DPPH assay and chromatography-mass spectrometry. Sharma et al. 2019 reported the mosquito-borne diseases such as malaria, filariasis, chikunguniya, yellow fever, dengue and Japanese encephalitis are the major cause of remarkable morbidity and mortality in livestock and humans worldwide. Natural insect repellents like essential oils has exhibited good efficacy against mosquitoes. It is a mixture of components including citronellal, citronellol, geraniol as major constituents contributing to various activities (antimicrobial, anthelmintic, antioxidant, anticonvulsant antitrypanosomal and wound healing), besides mosquito repellent action. Citronella essential oil is registered in US EPA (Environmental protection agency) as insect repellent due to its high efficacy, low toxicity and customer satisfaction.

Priyanka et al. 2018 reported turmeric oil was extracted from *Curcuma Longa* herb (turmeric root) using supercritical fluid extraction (SFE) process. Turmeric oil yield found through Soxhlet extraction was 5.954 wt% of turmeric powder whereas through SFE, it varied from 2 to 5.3 wt%. Turmerone and curcumin were identified as principle compounds of turmeric essential oil. Economic assessment of SFE of turmeric oil at industrial scale for 60 t/y production capacity was performed. Li et al. 2019 studied the preparation, characterization, anti-aflatoxigenic activity, and molecular mechanism in vitro of chitosan packaging films containing turmeric essential oil (TEO). The inhibitory effects of pure chitosan films and packaging films containing 1.5 μL/cm^2 or 3.0 μL/cm^2 TEO on the growth and conidial formation of *Aspergillus flavus* (*A. flavus*, CGMCC 3.4410), as well as the accumulation of aflatoxin over the course of seven days. The packaging films possessed a prominent antifungal activity on *A. flavus*. Gene expression of packaging films which inhibit aflatoxin biosynthesis. The expressions levels of 16 genes related to aflatoxin biosynthesis were found to be either completely or almost completely inhibited. Therefore, the addition of the natural antifungal agent TEO in chitosan packaging films represent a remarkable method to significantly promote the development and application of antifungal packaging materials. Ludmila et al. 2020 reported the packaging materials based on biopolymers are gaining increasing attention due to many advantages like biodegradability or existence of renewable sources. Grouping more antimicrobials agents in the same packaging can create a synergic effect, resulting in either a better antimicrobial activity against a wider spectrum of spoilage agents or a lower required quantity of antimicrobials. In this work, investigation of an antioxidant activity of essential oils of Lemon grass (*Cymbopogon flexuosus*), Turmeric oil (*Curcuma longa*) and Palmarosa (*Cymbopogon martinii*) was carried out and characterized with FTIR analysis.

2. Materials and Methods:

The antioxidant potential of essential oils was evaluated using the Ferric Reducing Antioxidant Power (FRAP) assay and DPPH radical scavenging.

2.1 Chemicals and Reagents

Hi-media provided the following products: linoleic acid, DPPH (2,2-diphenyl-1-picrylhydrazyl), β-carotene, ascorbic acid, ferric chloride, butylated hydroxytoluene (BHT), anhydrous sodium sulphate, menthol (≥99%), methanol, ethanol, potassium ferricyanide, trichloroacetic acid (TCA), chloroform, and dimethyl sulfoxide (DMSO). The following were acquired from molychem.ay and Nice: citral (≥96%), geraniol (≥98%), linalool (≥97%), α-bisabolol (≥95%), citronellol (≥95%), and citronellal (≥95%).

2.2 Distillation process

The raw materials for the essential oils that were steam-distilled came from the essential oil mills situated in the Thanippadi rural area of Thiruvannamalai district. The samples were distilled to get pure essential oil (Figure 2).

Figure 1: Diagram representing the extraction action of essential oil by using distillation process from Lemon grass (*Cymbopogon flexuosus*), Turmeric oil (*Curcuma longa*) and Palmarosa (*Cymbopogon martinii*)

2.3 Ferric Reducing Antioxidant Power

Ferric reducing antioxidant power (FRAP) was determined by Oyaizu (1986), with slight modification, Briefly, each 1.0 ml of essential oil solution containing different amount of essential oil (10,20,30,40,50 mg/ml was dissolved in DMSO to make 1.0 ml solution) were mixed with 2.5 ml of phosphate buffer (0.2 M, PH 6.6) and 1.0 ml of potassium ferricyanide (1%). The mixture was then incubated 50 ^{0}C for 20 min. afterwards, 2.5 ml of TCA (10 %) was added to mixture, which was then centrifuged at 6000 g for 5 min. Finally, 2.5 ml of supernatant was mixed with 2.5 ml of distilled water and 0.5 ml of ferric chloride (0.1%), after 10 min. the absorbance was measured at 700 nm. Same amount of DMSO was used in place of essential oil solution as blank. All the tests were

carried out in triplicate. Ascorbic acid as positive control. Higher absorbance of reaction mixture indicates greater reducing power.

2.4 DPPH radical scavenging assay

To quantify the DPPH radical scavenging activity of essential oils, we employed a slightly altered version of Blois's (1958) methodology. 0.9 ml of the different essential oil solution concentrations (100 µl) in methanol was mixed with a 100 µM DPPH solution. In comparison to the blank, the absorbance at 517 nm was measured following 30 minutes of dark incubation at 37 °C. There were three copies of every exam given. The same volume of methanol was added to the blank essential oil solution. We used ascorbic acid as a positive control. To calculate the percentage of radical scavenging activity, the following formula was used:

Percent radical scavenging activity = [(A0-A1)/A0] ×100

Where,

A0: absorbance of blank,

A1: absorbance of test solution with essential oil.

2.5 Fourier Transformed Infrared Spectroscopy (FTIR)

A study using FTIR spectroscopy was conducted to examine the structural characteristics of essential oils. A Perkin-Elmer FTIR spectrophotometer was used to record the spectra under ambient circumstances. FTIR spectrum analysis was conducted in the 400- 4000 cm^{-1} wave number range.

2.6 Antimicrobial Activity

The essential oils from Lemon grass (*Cymbopogon flexuosus*), Turmeric oil (*Curcuma longa*) and Palmarosa (*C. martinii*) were tested by disc diffusion method (Anonymous, 1996). About 100 µg/ml sample were used. The test microorganisms were seeded into respective medium by spread plate method. Muller Hinton Agar medium was prepared and poured into the petri dishes. After solidification the filter paper discs (5 mm in diameter) impregnated with the extract were placed on test organism-seeded plates. Chloremphenicol (10 µg/ml) used as positive control and essential oils (100 µg/ml) used as test sample. The antimicrobial assay plates were incubated at 37 ºC for 18 hours. The diameter of the inhibition zones was measured in millimeters (mm).

3. Results and Discussion

Fourier Transformed Infrared Spectroscopy (FTIR)

A study using FTIR spectroscopy was conducted to examine the structural characteristics of essential oils. A Perkin-Elmer FTIR spectrophotometer was used to record the spectra under ambient circumstances. The range of

wave numbers in which FTIR spectrum analysis was conducted was 400–4000 cm^{-1}. The spectrum of the essential oils was shown in figure 2. The bands with the strongest influence on the principal components in the group essential oils are bands at ~1375 cm^{-1} and 1450 cm^{-1}, a broad band at 3400–3500 cm^{-1}, in addition to a band at 842 cm^{-1} that was shifted to 862 cm^{-1} in essential oils. The vibrational frequencies for acetate functional group correctly in the vinyl group vibration RHC=CH_2 at 1635–1650 cm^{-1}, but the intensities of these peaks are very low. Peaks were found in the essential oils of over 200 plant species, belonging to different families (Stashenko et al.2008). Common essential and its ester linalyl acetate are the main constituents in lavender oil. The C=CH_2 in-plane deformation vibration occurs near 1420 cm^{-1}. Only linalool, linalyl acetate and myrcene show this band, while limonene and β-pinene show no trace of it. However, this band may be hidden under the CH_2 and CH_3 deformation and near 1450 cm^{-1}. The =CH_2 in plane deformation vibration was not found as a separate band near1410 cm^{-1}, and was most probably hidden under the−CH_3 and =CH_2 absorption bands. The presence of a =CH_2 group may also explain why the 1330–1410 cm^{-1} intensity is higher for some terpenes (Rummens, 1963).

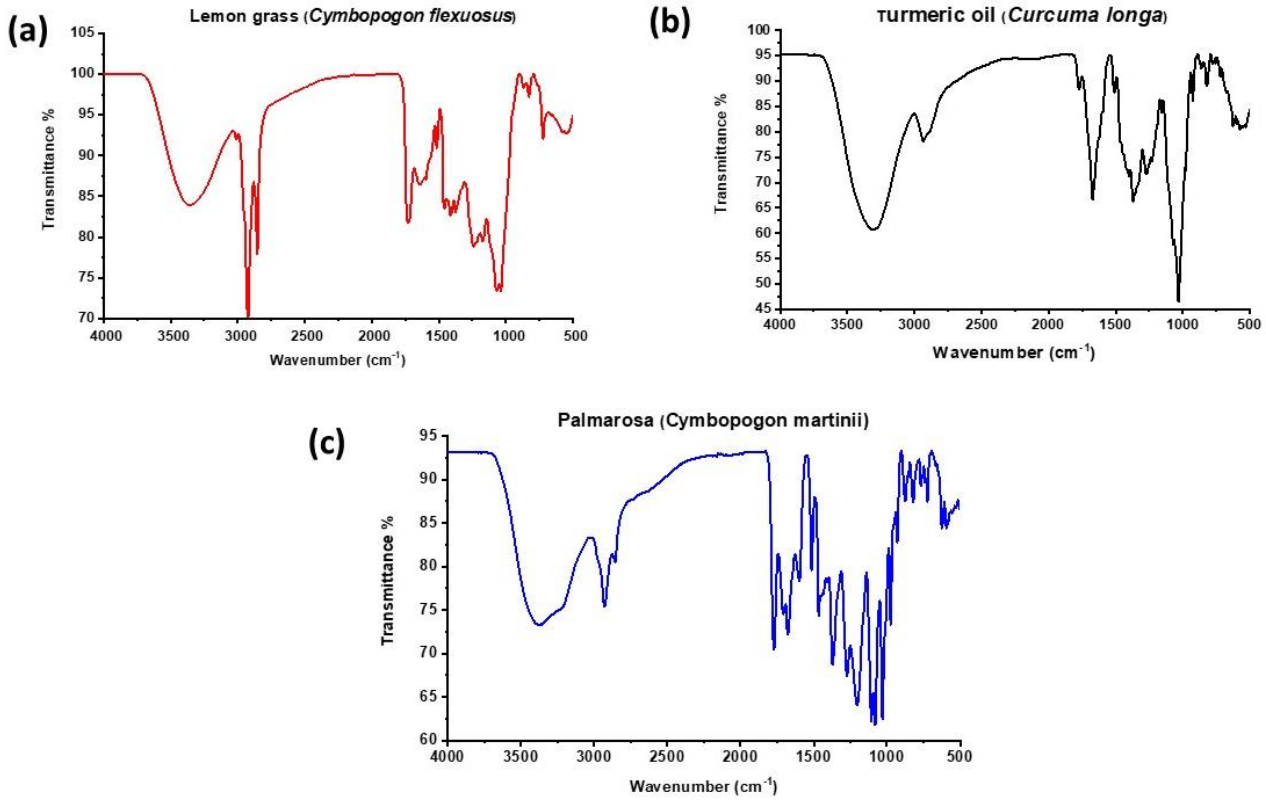

Figure 2: FTIR spectra of Lemon grass (*Cymbopogon flexuosus*), Turmeric oil (*Curcuma longa*) and Palmarosa (*Cymbopogon martinii*) for the region ranges from 4000 – 500 cm^{-1}

Antioxidant activity:

The essential oil of the Lemon grass (*Cymbopogon flexuosus*), Palmarosa (*C. martinii*), Turmeric oil (*Curcuma longa*) were obtained by hydrodistillation showed pale yellow and yellowish color with a good pleasant aromatic odor. Employing the two different types of methods antioxidant activity were investigated. The essential oil of *lemon grass* showed antioxidant activity in RPA (66.87%), DPPH (79.90%) with free radical-scavenging (Figure 8 & 9). Antioxidant activity of Palmarose oil were noticed for RPA (76.16%), DPPH (82.13) with activity (Figure 3

& 4) and also turmeric oil antioxidant for RPA (75.29%), DPPH (85.80) for the concentration at 50mg/mL. The highest antioxidant activities (85.80%) were obtained in turmeric oil for DPPH assay and (76.17%) for RPA assay for lemon grass oil. The essential oil showed an antioxidant activity in the Tables 1 & 2.

Table 1: Antioxidant activity of Lemon grass (*Cymbopogon flexuosus*), Turmeric oil (*Curcuma longa*) and Palmarosa (*Cymbopogon martinii*) by RPA assay

S. No.	Concentration of Essential Oil (µg/ml)	Reducing power assay for Lemon grass		Reducing power assay for Turmeric oil		Reducing power assay for Palmarosa	
1	10	0.384	41.75	0.384	43.02	0.910	40.44
2	20	0.351	45.91	0.351	57.36	0.697	54.38
3	30	0.316	51.30	0.316	63.71	0.586	61.64
4	40	0.281	56.70	0.281	74.70	0.399	73.38
5	50	0.215	66.87	0.215	75.29	0.364	76.17
6	Control	0.649		0.846		1.528	

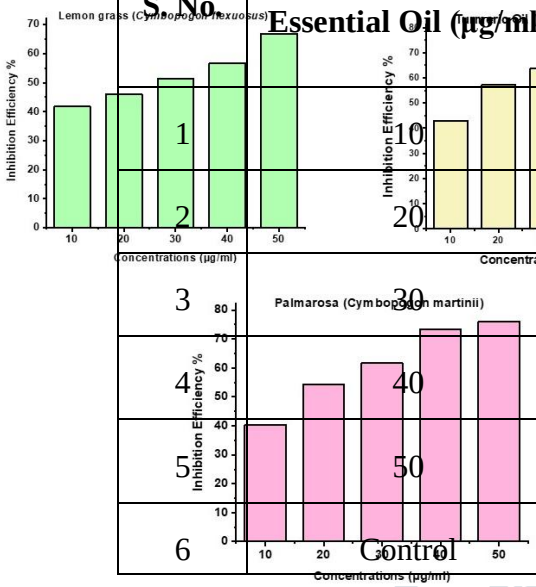

Figure 3: Bar chart representing antioxidant activity of Lemon grass (Cymbopogon flexuosus), Turmeric oil (Curcuma longa) and Palmarosa (Cymbopogon martinii) by RPA assay

Table 2: Antioxidant activity of Lemon grass (Cymbopogon flexuosus), Turmeric oil (Curcuma longa) and Palmarosa (Cymbopogon martinii) by DPPH assay

S. No.	Concentration of Essential Oil (µg/ml)	DPPH assay for Lemon grass		DPPH assay for Turmeric oil		DPPH assay for Palmarosa	
1	10	1.180	30.87	0.508	25.08	0.827	23.43
2	20	0.960	43.76	0.384	43.29	0.620	42.59

3	30	0.810	52.54	0.243	64.03	0.474	56.11
4	40	0.541	68.30	0.162	76.06	0.279	74.16
5	50	0.343	79.90	0.096	85.80	0.191	82.13
6	Control	0.663		0.678		1.08	

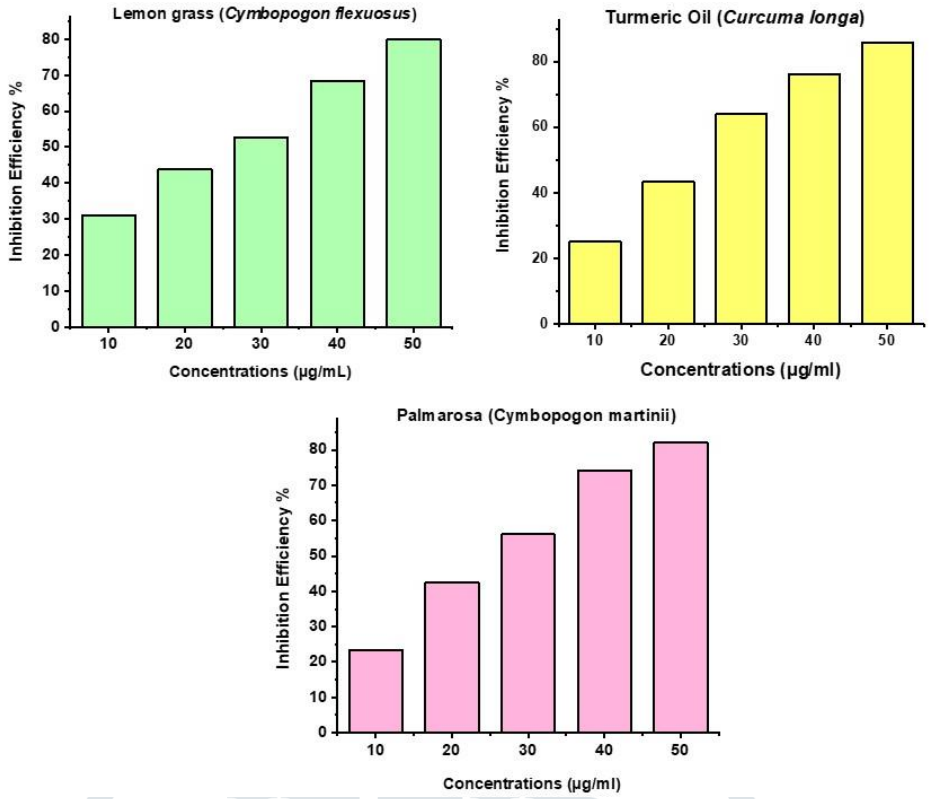

Figure 4: Bar chart representing antioxidant activity of Lemon grass (Cymbopogon flexuosus), Turmeric oil (Curcuma longa) and Palmarosa (Cymbopogon martinii) by DPPH assay

Antimicrobial Activity

The pure natural sources of essential oils are their strong medicinal and therapeutic qualities. We are now investigating the effectiveness of essential oils against common infections such as Aspergillus niger, Salmonella Typhi, and Escherichia coli. The concentration of the turmeric extract used was 100 (µg/mL). Figure 5 displayed this zone of inhibition results. The oil with the highest action is turmeric. Every essential oil has the capacity to eradicate fungi and germs. Salmonella Typhi (23 mm), Escherichia coli (TO-20 mm), and Aspergillus niger (TO-21 mm) are the three microorganisms.

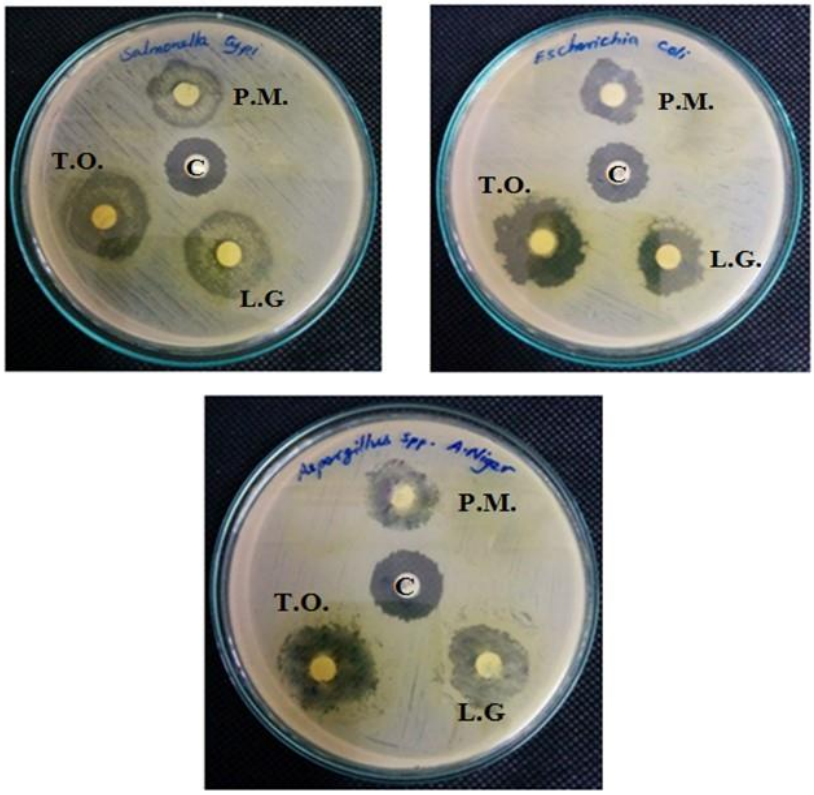

Figure 5: Antimicrobial activity of Lemon grass (Cymbopogon flexuosus), Turmeric oil (Curcuma longa) and Palmarosa (Cymbopogon martinii)

Table 3: Antimicrobial Activity of Essential Oils

S.NO.	Name of Organism	Control	Concentration of Essential Oil (100µg/mL)		
			Lemon grass	Turmeric oil	Palmarosa
1.	*Salmonella Typhi*	15	19	23	16
2.	*Escherichia coli*	16	19	20	15
3.	*Aspergillus niger*	16	18	21	16

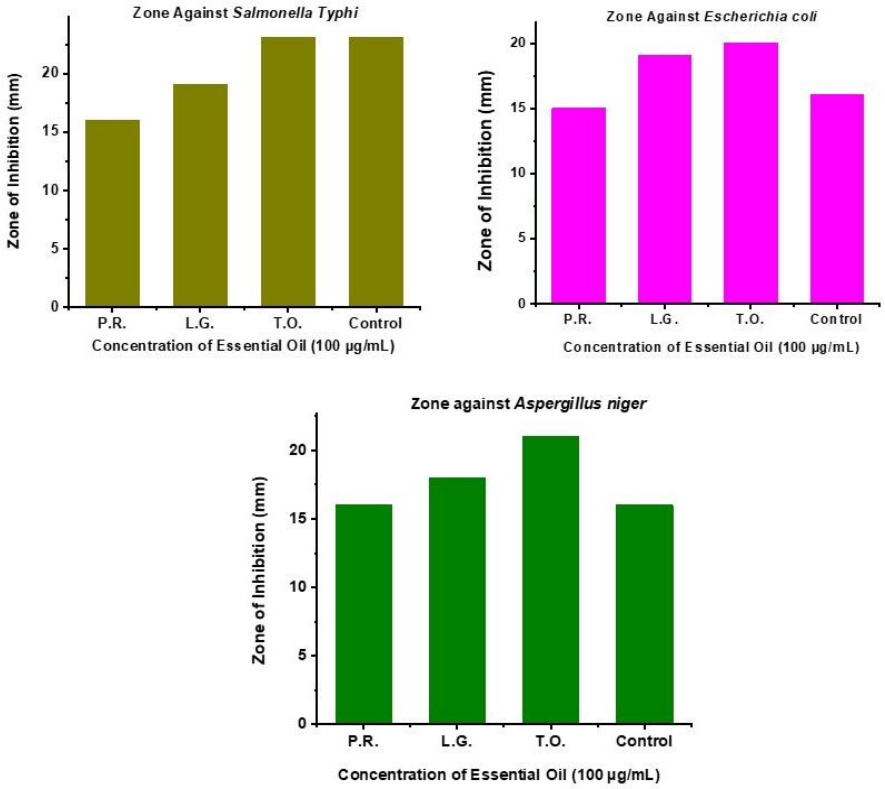

Figure 6: Bar chart representing antimicrobial activity of Lemon grass (Cymbopogon flexuosus), Turmeric oil (Curcuma longa) and Palmarosa (Cymbopogon martinii)

4. Conclusion

The antioxidant capability of each essential oil and each of its constituent parts was assessed. While we looked at two assays, all of the essential oils showed strong potential for antioxidants. Of these, the DPHH radical scavenging assay had a higher level of antioxidant activity than the reducing power assay. In comparison to those essential oils, the antioxidant capacity of all the other ingredients was low. The synergistic interaction of the oil components may account for the strong antioxidant capacity of essential oils. The results of this study indicate that these essential oils, as well as the individual plant components, have potential use as natural antioxidants. The research results show that turmeric oil and palmrose oil have more antioxidant potential than lemongrass oil. A thorough explanation was also provided regarding the FTIR characterization. It was concluded that the microbiological characteristics, essential oils have antimicrobial efficacy against *Salmonella Typhi*, *Escherichia coli*, and *Aspergillus niger*.

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