International Journal for Multidisciplinary Research (IJFMR)
E-ISSN: 2582-2160 ● Website: www.ijfmr.com ● Email: editor@ijfmr.com

Development of Herbal Surface Disinfectant using Essential Oil of Tea Tree Leaf

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Abstract:
Essential oils are concentrated hydrophobic liquids containing volatile compounds. These oils are extracted from medicinal plants which have been used as a therapeutic agent. Essential oils are the extracted compound of the characteristic fragrance of medicinal plants and some ornamental plants. These oils are mainly used as aromatherapy and have a healing effect. These oils were primarily used as medicines in the ancient period which was very useful according to some research papers. Essential oils can also contain an enormous amount of antimicrobial activity which is used to inhibit the growth of microorganisms that are life-threatening. The Antimicrobial activity of Essential oil can be used as a disinfectant which can kill or inhibit the growth of microbes. Places such as Hospitals and healthcare centers are prone to nosocomial infections which are very dangerous and threatening to patients as well as visitors. To prevent such outbreaks of infections the health care centers and hospitals are disinfected and cleaned thoroughly and it has been a regular practice. Replacing the disinfectants with the essential oils that have such antimicrobial activity will be completely new to the organisms. The essential oil of Melaleuca alternifolia [tea tree oil (TTO)] has been used medicinally for about 80 years.(9) TTO has broad-spectrum antimicrobial9 and anti-inflammatory(10,11) activity in vitro. Hammer et al.12 showed that transient skin organisms were more susceptible to TTO than commensal organisms. This finding supports the use of TTO-containing handwash products since normal skin flora represents one of the natural defenses against colonization by pathogenic organisms. Other reports have suggested that the repeated use of TTO-containing hand wash does not lead to dermatological problems associated with some formulations,14 and this finding might be used to encourage healthcare staff's compliance with handwashing. Although the antibacterial activity of TTO has been well-established in vitro. TTO has not yet been assessed using European standard methods that are now widely accepted for the evaluation of disinfectant and antiseptic efficacy. In this study, we assessed the activity of TTO and TTO-containing formulations according to two European standard suspension methods, EN 127620 and prEN 12054.

1. INTRODUCTION

Essential oils are obtained from plants and are aromatic because of a mixture of diverse chemical substances. They have tremendous business potential on the global market due to their unique flavor, fragrance properties, and biological activities.

Essential oils (EOs) are oily, aromatic, and volatile liquids that can be harvested from plant material. Usually, EOs are formed in specialized cells or groups within stems or leaves and are concentrated in particular regions of the plant, such as the bark, leaves, or fruit. The main components are hydrocarbons (pinene, limonene, and bisabolene), alcohols (linalool and sotalol), acids (benzoic acid and geranic acid), aldehydes (citral), cyclic aldehydes (cuminal).

Essential oils are multifunctional and exhibit various activities, such as antiphlogistic, spasmolytic, antinociceptive, immunomodulatory, psychotropic, acaricidal, expectorative, and cancer-suppressing activities. Furthermore, EOs and their components possess antibacterial, antifungal, antiviral, insecticidal, and antioxidant properties.

In medicine, only a few EOs are used in aromatherapies and some components of EOs are used for flavoring in the food industry. Essential oils have already found a considerable range of applications. The majority of them are used as fragrances in perfumery as well as in the food and beverages industry. The recent years have also witnessed a revival of traditional natural products in medicine and in food and cosmetics preservation. Despite the development of antibiotics, bacterial and fungal infections are still a major issue in medicine, and the presence of numerous drug-resistant strains poses a new challenge.

In this regard, plant essential oils may offer great potential and hope. The most recent reviews are non-English ones [5-7]. This paper gives an overview of the activity of essential oils derived from various plants as well as their wide variety of methods used and antimicrobial activity concerning the possible application of essential oils in medicine and food, cosmetic, and pharmaceutical industries

Several researchers have recognized the antimicrobial impacts of essential oils and their chemical components in the past.

These oils are extracted from medicinal plants which have been used as therapeutic agents. They are used in aromatherapy, skincare, and sometimes in cooking. Each oil has unique properties and purported benefits, but it's important to use them safely and be aware of potential allergies or sensitivities. Two serious problems, the development of resistance by plant pathogenic fungi & bacteria and the presence of high-level toxic residues in agricultural products hamper the effective use of chemical fungicides and bactericides in controlling plant pathogenic microbes. Hence the exploitation of natural substances such as essential oils, safer to consumers and the environment, for the control of plant diseases is presently looked upon.

The ventilation performance, dust loading conditions, and biological contaminants all contribute to the air quality. Most biological contaminants, such as bacteria, molds, and yeasts, are categorized as potentially allergenic .Continuous exposure to these biological contaminants can lead to irritation, allergies, and infections . Essential oils are the extracted compounds of the characteristic fragrances of medicinal plants and some ornamental plants. These oils are mainly used as aromatherapy and have a healing effect. These oils were primarily used as medicines in the ancient period which was very useful according to some research papers. Essential oils can also contain an enormous amount of antimicrobial activity which is used to inhibit the growth of life-threatening microorganisms.

The Antimicrobial activity of Essential oil can be used as a disinfectant which can kill or inhibit the growth of microbes. Places such as Hospitals and healthcare centers are prone to nosocomial infections which are very dangerous and threatening to the patients as well as the visitors. To prevent such outbreaks of infections the health care centers and hospitals are disinfected and cleaned thoroughly and it has been a regular practice. They are replacing the disinfectants with essential oils which have antimicrobial activity and will be completely new to the organisms. Nosocomial infections are one of the most infectious diseases which are the main reasons for many outbreaks of diseases.

In most healthcare centers and hospitals, they tend to use disinfectants to clean surfaces and other areas. Exposure to these harmful chemical disinfectants can cause irritants to the skin, eyes, and respiratory system. They are also corrosive so they could affect the instruments and machines. It requires many

precautions while using. Replacing this with herbal disinfectants can reduce the irritations and they are not corrosive.

The purpose of using natural plant material as an herbal disinfectant is to reduce the risk of unwanted effects of chemical disinfectants. Essential oil extracted from the tea tree leaves is purchased commercially and incorporated with the disinfectant-making process and compared with the other chemical disinfectants to check the ability to kill the microorganisms on the surfaces and floors.

The essential oil of *Melaleuca alternifolia* [tea tree oil (TTO)] has been used medicinally for about 80 years.. This finding supports the use of TTO-containing handwash products since normal skin flora represents one of the natural defenses against colonization by pathogenic organisms. Although the antibacterial activity of TTO has been well established in vitro, TTO has not yet been assessed using European standard methods that are now widely accepted for the evaluation of disinfectant and antiseptic efficacy. In this study, we assessed the activity of TTO and TTO-containing formulations according to two European standard suspension methods, EN 127620 and prEN 12054.

2. AIM AND OBJECTIVE

The aim of the study is to:

- Develop a potential natural disinfectant as an alternative to chemical disinfectants.

The objectives are :

- To analyze the antibacterial and antifungal activity of the tea tree oil.
- To produce a disinfectant that can suppress the activity of bacteria and fungi.
- Performing the standard test procedure for efficient disinfectant

3. REVIEW OF LITERATURE

The oil of *Melaleuca alternifolia*, also known as tea tree oil (TTO), has been used as an antiseptic remedy for decades. Although there is no published documentation of specific medicinal applications of the *M. alternifolia* plant or oil by Aboriginals before white colonization of Australia. Extensive medicinal use of TTO did not begin until its antiseptic and disinfectant properties were reported in the 1920s by Penfold and Grant.3 It has been claimed that TTO was used by Australian munitions factories during World War II. Also during the war, maintaining production of TTO was considered so vital that bush cutters of *M. alternifolia* were exempt from national service.

A study in 1990 examining the effect of a 5% TTO product versus 5% benzoyl peroxide for the treatment of acne revealed that TTO and benzoyl peroxide were equally effective in reducing acne lesions, although TTO use resulted in fewer side effects(6) Since then, a wide variety of TTO products have been formulated, and further studies on the effects of TTO against a broad range of microorganisms and superficial clinical conditions have been conducted. One such study has demonstrated that hand washes containing 5% TTO are more effective at removing contaminating bacteria from hands than regular nonmedicated soap. (11) Despite the popularity of TTO and TTO products, only very few studies of the appropriateness and in vitro efficacy of commercial TTO formulations that claim to have antiseptic activity have been undertaken. (11) In two of these studies, the release of terpinen-4-ol from several different topical formulations was found to depend on both the formulation of the preparation and the concentration of TTO(12,13) This is particularly important since terpinen-4-ol has been claimed to be one of the main components responsible for the antimicrobial activity of TTO(14,15) Other natural products that have shown antimicrobial activity, such as propolis, have been tested for synergism with topical antimicrobials

or compared to standard treatments with good outcomes(16,17) When antiseptic TTO products are formulated, the activity of the preparations should be attributed to the active ingredient, namely, the TTO. Having a product with optimal TTO solubility in the base and optimal delivery of TTO to the affected skin area in appropriate concentrations must be considered paramount for marketing a successful antiseptic TTO preparation. The potential exists for poorly formulated products, where the antiseptic activity of TTO has been supplemented by the presence of preservatives such as parabens, or co-solvents such as alcohols that have antimicrobial activity themselves.

For this reason, TTO is largely employed in Australia, Europe, and North America as an active component in hand-cleansing formulations, shampoos, and topical preparations for the treatment of cutaneous infections and other ailments. There are six naturally occurring chemotypes in *Melaleuca alternifolia* each one producing oil with a distinct chemical composition and identified by the presence of terpinen-4-ol, cineol, or terpinolene [1, 18, 21].

To date, studies about the efficacy, safety, and uses of TTO have been performed using the terpinene-4-ol chemotype which has been consistently identified as the most effective antimicrobial component [1]. In the terpinen-4-ol chemotype, most of the antimicrobial activity is ascribed to the 30–40% in content of terpinen-4-ol, but the analysis of over 800 terpinen-4-ol TTOs identified more than 100 components at different ranges of concentrations [4].

The International Standard Organization (ISO 4730:2017) regulates the concentration ranges of the major TTO terpenes and related alcohols and ethers [19]. However, because of the inherent variability of chemotypes, dissimilarity in preparation, and different duration of storage, batch-to-batch variations and different antimicrobial efficacy have been reported in commercially available TTOs [24].

3.1 CHEMICAL COMPOSITION OF ESSENTIAL OIL

Almost 100 types of components in TTO have been identified [57]. It was found that 50% of them are hydrocarbons and 50% are oxygenated compounds. The variation characterizes essential oils from different geographical sources. However, the predominant compounds are monoterpenes, sesquiterpenes, and their respective alcohols. Melaleuca species present a multitude of aromatic compounds in their volatile oils, including monoterpenoids such as terpinen-4-ol, terpinolene, p-cymene, α-terpineol, 1,8-cineole, α-pinene, and α-terpinene. Sesquiterpenoids in these oils are β-caryophyllene, ledol, (E)-nerolidol, viridiflorol, monoterpene, and alcohol terpineol. Phenylpropanoids in TTO are methyl eugenol and methyl isoeugenol.

The most investigated species of Melaleuca for ornamental purposes and essential oil production is Melaleuca alternifolia. Under natural conditions, it grows up to 8 m; 3-year-old trees produce whitish- or cream-colored flowers as terminal spikes. The oil from this species is of commercial interest and is utilized by different industries. The main components of this oil are hydrocarbons of terpenes which include terpinen4-ol, alpha and gamma terpinene, 1,8-cineloe, and terpinolene. These are chemical compounds with the formula C5H8, having a volatile and aromatic nature. In the second half of the 20th century, various compounds have been identified in TTO by different analytical methods. Gas chromatography and GC with mass spectrometry revealed about 100 compounds, with terpinen-4-ol being the chief component accounting for 40% of the total composition. Terpinene and α-terpinene were the second and the third most concentrated compounds, representing 23% and 10% of the total content, respectively. The other main compounds in that oil were identified as 1,8-cineole, terpinolene, p-cymene, α-pinene, α-terpinolene, aromadendrene, limonene, and sabinene [52]. The storage conditions (heat and air exposure, light, and moisture) can alter the chemical composition of TTO, as some compounds such as alpha and gamma-

terpinene may degrade with time. Therefore, the oil must be stored in a cool, dry, and dark place, most likely in an oil veil to prevent chemical changes. Terpinen-4-ol was shown to inhibit inflammation in activated human monocytes. When tea tree oil and terpinen-4-ol were evaluated on human melanoma cells, they blocked their growth and promoted their death. in Table 1, the compounds identified in the oil in various studies are listed.

Table 1. Compounds extracted from M. alternifolia, as reported in different studies.

Identified Compounds	Quantity (%) [63]	Quantity (%) Miscellenious Studies
Sabinene	0.41	0.2 [52]
Alpha-Pinene	1.66	2.67 [64]
I-beta-Pinene	0.49	0.3 [52]
β-Pinene	0.24	0.71 [65]
α-Terpinene	9.09	7.69 [64]
Eucalyptol	5.03	-
γ-Terpinene	22.66	19.54 [64]
Terpinolene	1.76	3.09 [64]
Terpinen-4-ol	53.98	40.44 [64]
α-Gurjunene	0.30	0.2 [52]
(+)-Gurjunene	0.86	-
Aromadendrene	0.31	1.5 [52]
δ-Cardinene	0.28	1.3 [52]
β -Gurjunene	1.73	0.1 [52]
δ-Cardinene	1.22%	1.3 [52]

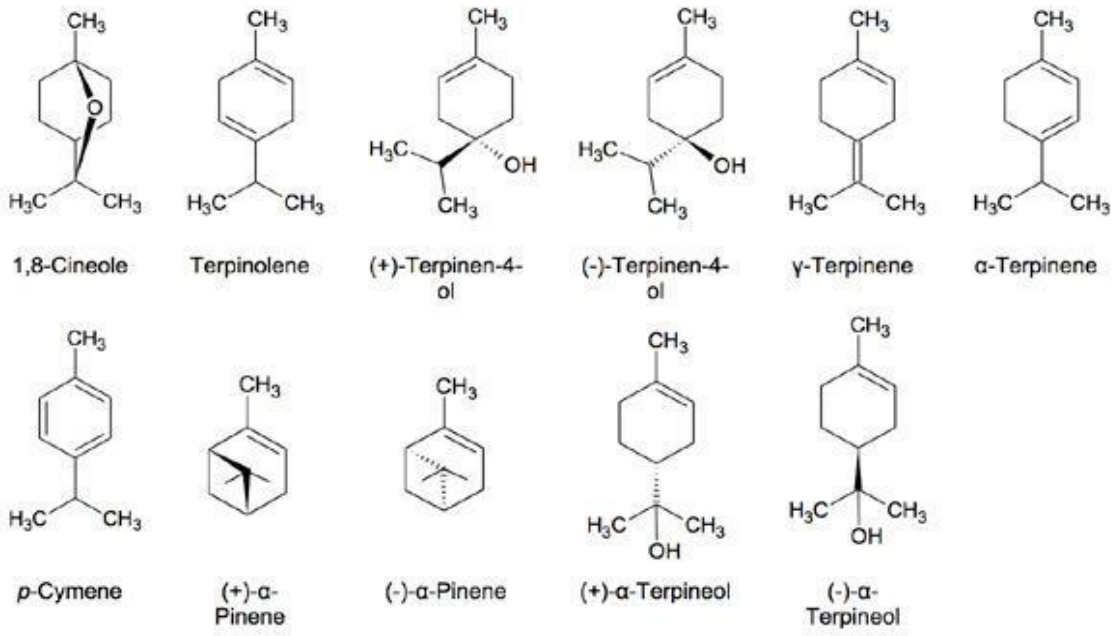

Figure 1. Major monoterpenes in tea tree oil

4. MATERIALS AND METHODS

4.1 Collection of oil sample

The oil sample *Melaleuca alternifolia* (tea tree)oil was purchased from the supermarket in Coimbatore.

4.2 Collection of Clinical Pathogens

The clinical pathogens such as *Escherichia coli, Staphylococcus aureus, Salmonella sp., Bacillus sp.,* and *Pseudomonas sp.,* cultures were collected from the KMCH hospital, Sitra, Coimbatore.

4.3 ANTIBACTERIAL ANALYSIS

4.3.1 Preparation of broth culture

All five clinical pathogens (*Escherichia coli, Staphylococcus aureus, Salmonella sp., Bacillus sp.,* and *Pseudomonas sp.,*) were cultured in Nutrient broth and grown overnight at 37°c for 24 hours, it was used in the antibacterial analysis.

4.3.2 Media preparation

Mueller Hinton Agar (MHA) composition (grams/L)

HM infusion B form - 300.00
Acicase - 17.50
Starch - 1.50
Agar - 17.00
Final pH - 7.3 ± 0.1

38 grams of MHA (Mueller Hinton Agar) was dissolved in 1000ml of distilled water and then autoclaved for 15 minutes at 121°c. Once the medium was about 45°c - 50°c, it was poured into sterile Petri dishes. Then it was allowed to set completely.

4.3.3 Antibacterial activity

Antibacterial activity was performed by the agar well diffusion method. The 20µl of overnight broth culture was swabbed on MHA plates with sterile cotton swabs and allowed the plates for 2-3 minutes. The well was punctured with a well cutter, and the 30mul of TTO extract was loaded in a well. Penicillin (6mm/disc) was used as a positive control. The plates were incubated at 37°c for 24 hours. After incubation, the diameter of zone inhibition (mm) was measured and recorded.

4.3.4 Determination of minimum inhibitory concentration (MIC) of oil

The MIC was defined as the lowest concentration that completely inhibited the growth for 24 hours (Thongson et al., 2004). The MIC for the oil was determined by the agar well diffusion method. Tea tree oil was then diluted in sterile DMSO to achieve a decreasing concentration of 0.5 to 2.5%. A 50µl volume of each dilution was added aseptically into the wells in Mueller Hinton agar plates that had been inoculated at 37°c for 24 hours. Sodium propionate only served as a positive control. The lowest concentration of tea tree oil showing a clear zone of inhibition was considered as the MIC.

4.4 ANTIFUNGAL ACTIVITY

4.4.1 Collection of fungal specimens

The fungal cultures such as *Aspergillus niger, and Aspergillus flavus* were isolated from plates in Sabouraud's Dextrose Agar.

4.4.2 Antifungal activity

All the fungal specimens were cultured in Sabouraud's Dextrose broth growing at 28°c for 3 days, it was

used in antifungal analysis.

4.4.3 Media preparation

SD broth composition (gram/l)

Dextrose (glucose)	20.000
Peptone, special	10.000
Final pH (at 25c)	5.6 ± 0.2

30 grams of SD broth was dissolved in 1000ml of distilled water and then autoclaved for 15 minutes at 121°c. Once the medium was above 45°c to 50°c, it was poured into sterile petri dishes. Then it was allowed to set completely.

4.4.4 Antifungal activity

Antifungal activity was performed by the agar well diffusion method. The 15µl of broth culture was swabbed on SDA plates with sterile cotton swabs and allowed the plates for 2-3 minutes. The well was punctured with a well cutter then 50µl of oil was loaded onto the wells. Sodium propionate was used as a positive control. DMSO used negative control. The plates were incubated at room temperature at 28°c for 3 days. After incubation the diameter of the zone of inhibition (mm) was measured and recorded.

4.5 PRODUCTION OF DISINFECTANT

The production of the disinfectant using essential oil was made with alcohol ;

4.5.1 Materials required

- A transparent 8 oz bottle
- 15 drops of essential oil
- 1 & ¼ cups of white vinegar
- 1 & ¼ cups of water
- ¾ teaspoons of hydrogen peroxide
- ¼ cup of alcohol (60%)
- Funnel
- Mixing bowl

The disinfectant was made by adding all the materials mentioned above and was tested under the standard EN 1276.

4.6 TESTING METHOD OF THE DISINFECTANT

To evaluate *Melaleuca alternifolia*, essential oils as a disinfectant, the European standard EN 1276 (1997) and EN 1275 (1997) methods were used employing the standard bacterial and fungal strains under dirty conditions that are representative of surfaces which are known to or may contain organic and inorganic materials (3 g l)1 bovine albumin, 300 mg kg)1 CaCO3). The method of dilution-neutralization was employed, using (3% Tween80, 3% Saponin, 0Æ1% Histidine, and 0Æ1% Cysteine) autoclaved solution as a neutralizer. The product test concentrations were 0.5%, 0.75%, and 1% (v/v) for bactericidal activity and 0.5%, 1%, and 2% (v/v) for fungicidal activity. The contact time and test temperature were t = 5 min ± 10 s and h = 20 ± 1C, respectively.

4.6.1 Suspension test EN 12762

As described in the standard method, the formulations under test, 100% HSW, 100% AHSW, and, as controls, 5% TTO in 0.001% Tween 80 and 7.5% PVI were diluted to 55% (v/v) before testing. AHR (100%) was not diluted.

4.6.2 Neutralizer

The neutralizing solution used to quench the activity of antiseptics was based on European standard EN 127620 and contained: 30 g/L Tween 80 (Sigma), 3 g/L lecithin (Sigma), 1 g/L histidine (Sigma), 5 g/L sodium thiosulphate (BDH) and 34 g/L potassium dihydrogen phosphate (BDH) in tryptone soya broth (Oxoid).

4.6.3 Dilution-neutralization validation

The ability of the neutralizer to quench the corresponding antiseptic was assessed as follows: 1 mL of SDW was mixed with 1 mL diluent. Eight millilitres of antiseptic solution was added to this mixture and after 1 min, 1 mL was transferred into a test tube containing 8 mL of neutralizer. After 5 min, 1 mL of bacterial solution was added to the mixture and left in contact for 30 min. The final mixture was then serially diluted and counted using the DCM.

4.6.4 Interfering substances

As recommended by EN 1276, the interfering substance tested was a bovine albumin solution, under clean [0.3 g/L bovine albumin] or dirty (3 g/L bovine albumin) conditions. When the antiseptics were assessed in 'perfect' conditions.

4.6.5 EN 1276 suspension test

This quantitative suspension test method was designed for the evaluation of the bactericidal activity of chemical disinfectants and antiseptics used in food, domestic, and industrial areas. The requirement of this standard is a minimum reduction by a factor of 105 within 5 min. The antiseptic formulations were also assessed after a 1-min contact time, to better reflect real-life conditions.

One milliliter of interfering substance was mixed with 1 mL of bacterial test suspension. After 2 min, 8 mL of one of the product solutions was added to the mixture and shaken. After 1 and 5 min, 1 mL of the test mixture was transferred into a tube containing 8 mL of neutralizer and 1 mL of BSA, and mixed. The DCM was used as log10 reductions cannot be measured with the counting method recommended by the European standard. Plates were incubated for 24 h at 37°C, counted, and then re-incubated for a further 24 h to detect slow-growing colonies.

In this study, the aim was to compare the activity of:

(i) 5% TTO in Tween 80 with that of 7.5% PVI, 100% HSW, 100% AHSW, and 100% AHR in perfect, clean, and dirty conditions;

(ii) different concentrations of TTO in 0.001% Tween 80;

(iii) 5% TTO in Tween 80 with that of 0.001% Tween 80 alone; (iv) 100% HSW with that of 100% HSW without TTO; and

(v) 100% AHR with that of 100% AHR without TTO.

4.6.6 EN 1276 Test Conditions

- Test temperature – In between 4 °C to 60 °C
- Contact time – In between 1 min to 60 min
- Interfering substance – It is added in a test to evaluate the efficacy of the test material against other dirty or clean conditions that coexist with microbes in natural environments. Bovine serum albumin (BSA) is used as an interfering substance. (Clean Condition: 0.3g/l and Dirty condition: 3g/l of Bovine Serum Albumin).

4.6.7 Procedure for EN 1276 Test Method

- A sample of the product is added to a test suspension of bacteria in a solution of an interfering substance.

- This test suspension is kept for incubation under conditions (temperature and time) specified by the manufacturer.
- At the end of contact time, an aliquot is taken and immediately added to the neutralizing solution to suppress its bactericidal activity.
- Following a 5-minute neutralization period, 1 ml of the test mixture is plated onto Tryptone Soya Agar to detect surviving test bacteria.Plates are incubated for 20 – 24 hrs and the reduction in CFU (colony forming units) is calculated.

5. RESULT AND DISCUSSION

5.1 COLLECTION OF SAMPLE

PLATE 1: ESSENTIAL OIL

Fig 1: The tea tree leaf essential was purchased from supermarket

5.2 PREPARATION OF BROTH CULTURE

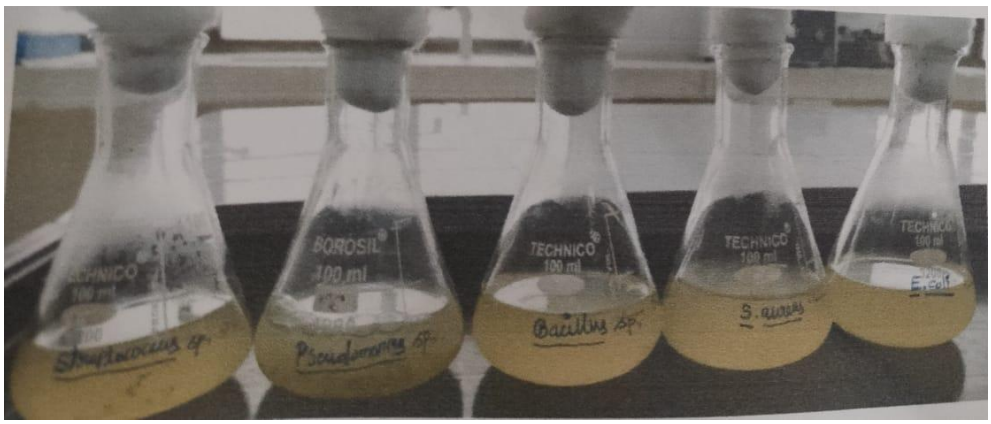

PLATE 2: BACTERIAL CULTURES

Fig 2: The broth cultures were prepared for the bacteria samples collected.

5.3 ANTIBACTERIAL ACTIVITY OF TTO

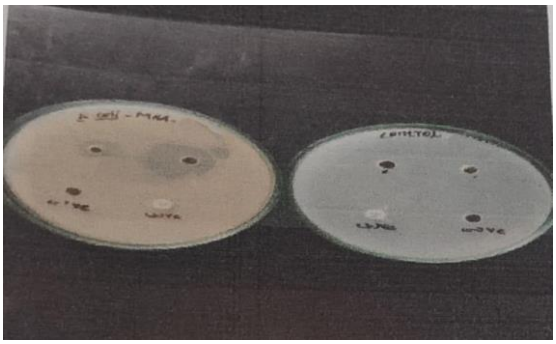

PLATE 3: ***Escherichia coli***

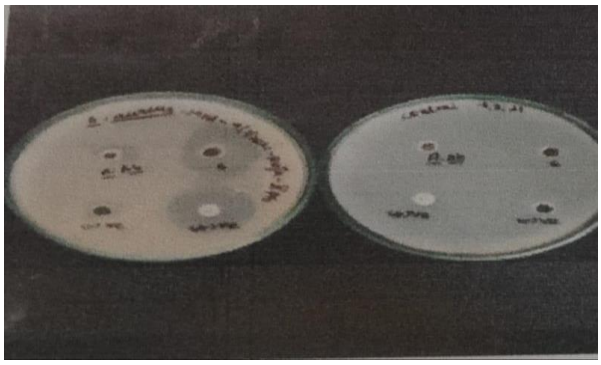

PLATE 4: ***Staphylococcus aureus***

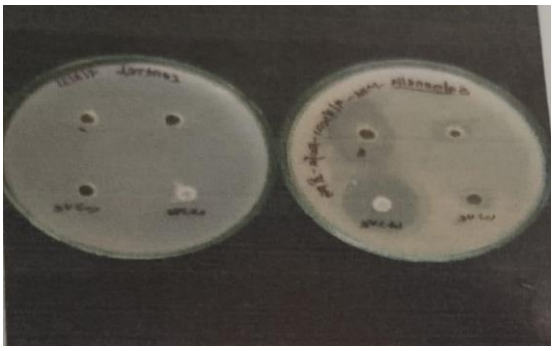

PLATE 5: ***Salmonella sp.,***

PLATE 6: ***Bacillus sp.,***

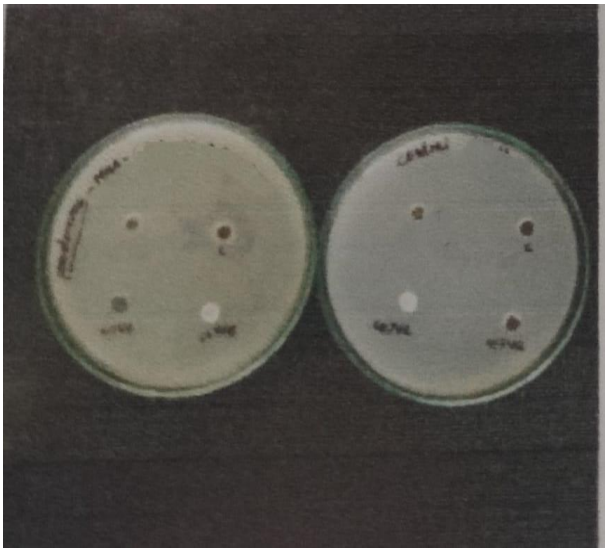

PLATE 7: ***Pseudomonas sp.,***

Fig 3: The plates inoculated with the bacterial cultures were observed for the zone of inhibition

5.4 MINIMUM INHIBITORY CONCENTRATION OF TTO

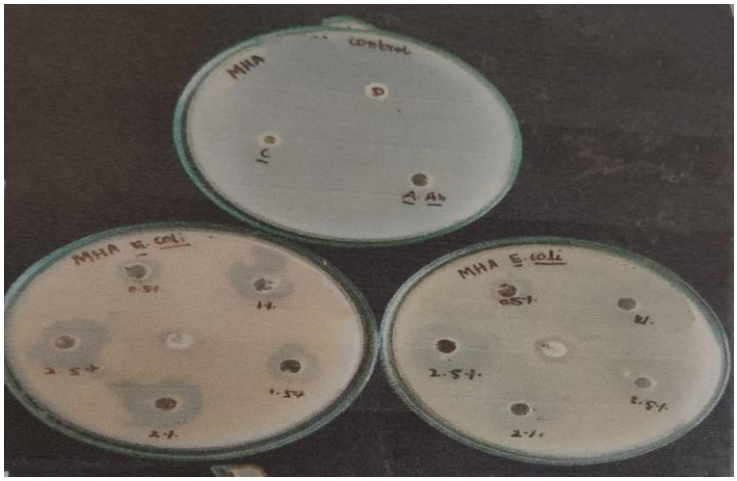

PLATE 8: ***Escherichia coli***

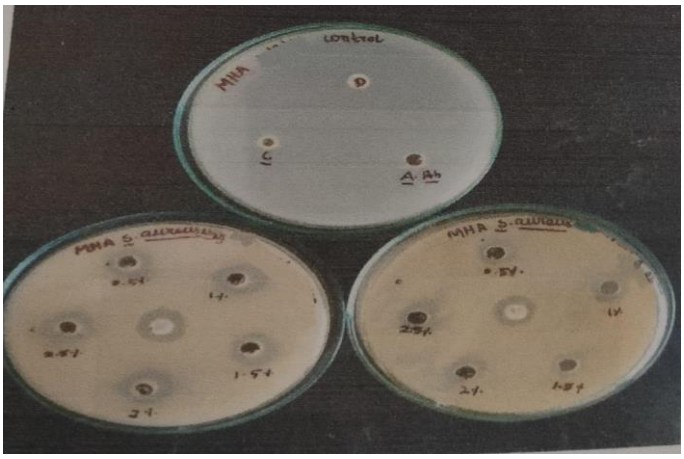

PLATE 9: Staphylococcus aureus

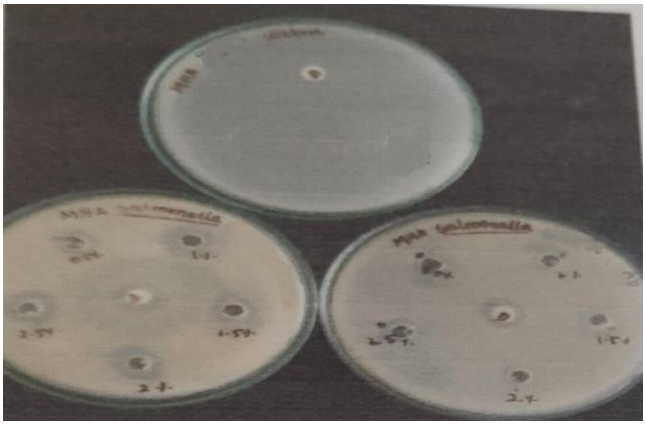

PLATE 10: ***Salmonella sp.,***

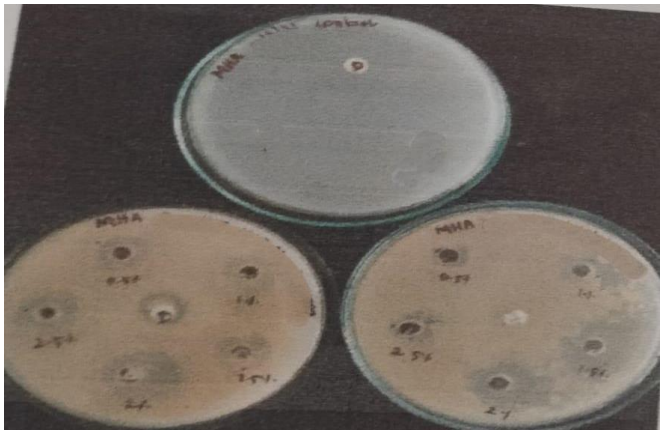

PLATE 11: ***Pseudomonas sp.,***

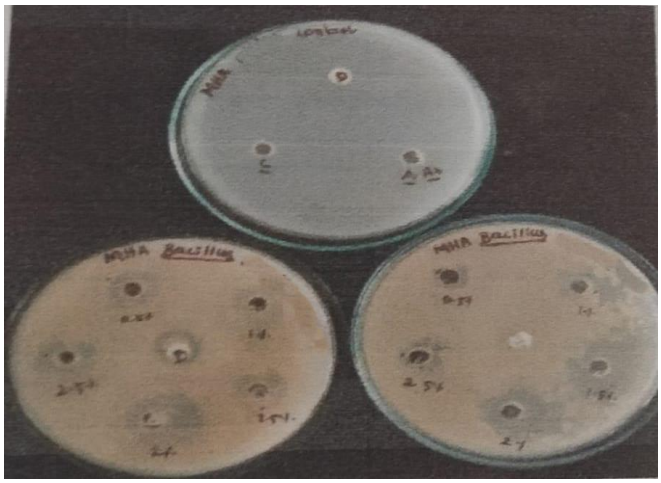

PLATE 12: ***Bacillus sp.,***

FIG 4: The minimum inhibitory concentration was observed for all the bacterial cultures collected.

5.5 ANTIFUNGAL ACTIVITY OF TTO

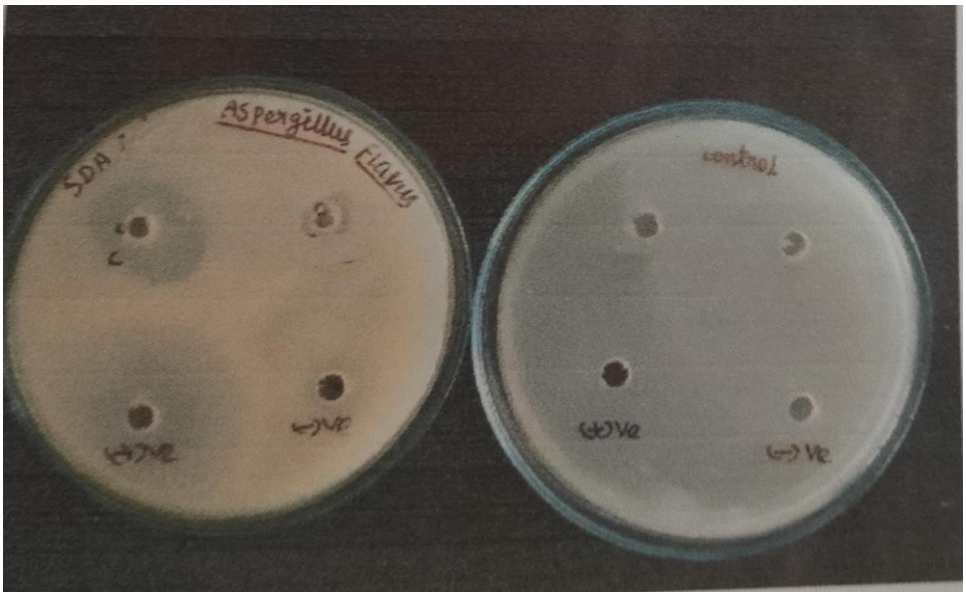

PLATE 13: ***Aspergillus flavus***

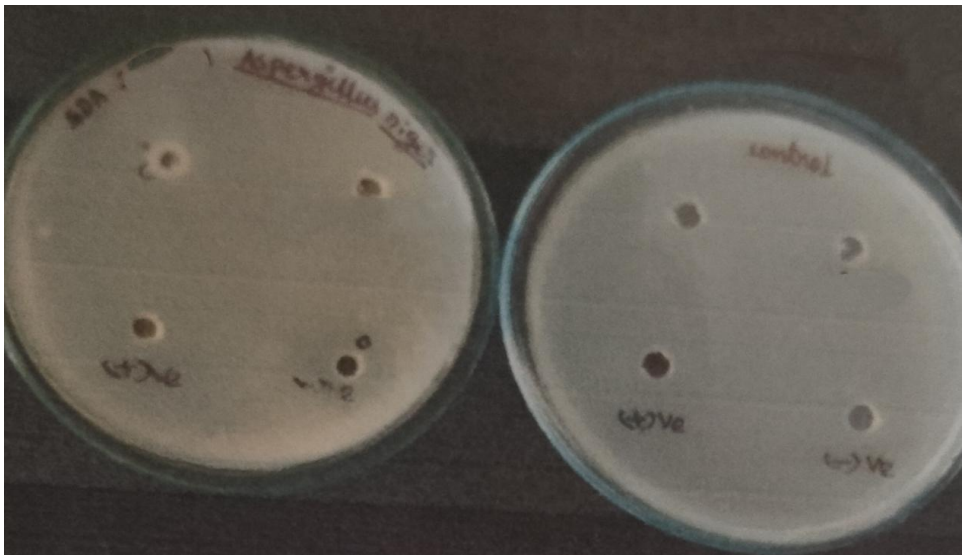

PLATE 14: ***Aspergillus niger***

Fig 5: The antifungal activity was observed for essential oil.

The essential oil of the Melaleuca genus has been utilized by Australia mainly because of its antimicrobial, anti-inflammatory, and anti-candidal traits. In tea tree oil (mainly from *Melaleuca alternifolia*), the major components terpinen-4-ol and 1,8-cineole are the main antimicrobial agents. The volatile oil from Melaleuca species at a 5% concentration had also passed the Therapeutic Goods ACT for antiseptics and disinfectants. Since the last decade, many studies and reports have been published to support the strong and wide-spectrum antimicrobial, antiseptic, and disinfectant potentials of tea tree oil as shown in Table 1.

Table 2 Antimicrobial spectrum of *Melaleuca alternifolia*

Microorganisms tested	MIC	MBC/MFC	Standard
Escherichia coli	0.08–2	0.25–4	0.32
Staphylococcus aureus	0.63–1.25	1.01	0.01
Salmonella sp.,	6.2	1.5	2.0
Pseudomonas sp.,	0.5	-	0.32
Bacillus sp.,	0.3	-	-
Aspergillus flavus	0.31–0.7	2–4	3.12
Aspergillus niger	0.3–0.4	2–8	>2.00

5.6 PRODUCTION OF DISINFECTANT

PLATE 15: DISINFECTANT

5.7 EN 1276 TEST METHOD

A sample of the product is added to a test suspension of bacteria in a solution of an interfering substance. This test suspension is kept for incubation under conditions (temperature and time) specified by the manufacturer. At the end of contact time, an aliquot is taken and immediately added to the neutralizing solution to suppress its bactericidal activity.

Following a 5-minute neutralization period, 1 ml of the test mixture is plated onto Tryptone Soya Agar to detect surviving test bacteria. Plates are incubated for 20 – 24 hrs and the reduction in CFU (colony forming units) was calculated.

The plates were observed under colony counter and the plate of 10^6 dilution yielded about 100 cfu/ml which is considered as the lowest possible level of detection of bacteria in a sample.

6. SUMMARY AND CONCLUSION

Based on the obtained results, it can be concluded that tea tree essential oil is richest in terpinene-4-ol, In addition to the main bioactive compounds, the results of our research showed that the essential oil of tea tree is rich in α-pinene (18.38%), limonene (7.55%), and γ-terpinene (14.01%).

The research showed tea tree essential oil's antimicrobial activity towards *Escherichia coli, Staphylococcus aureus, Salmonella sp., Bacillus sp.*, and *Pseudomonas sp.*, was observed efficiently and the antifungal activity towards *Aspergillus niger* and *Aspergillus flavus* was also somewhat efficient.

The determination of CFU is done by totaling the bacterial plaques that are present on an agar plate. Further, after this, the measurement of the total count of plaques that are there per unit volume in the original shock can be done. For ensuring that the calculation of CFU is precise, it can be ideal for counting between thirty and three hundred distinguished colonies on a typical ten-centimeter agar plate. By performing this test procedure we could say that the disinfectant is effective.

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