

Foot spray journal: Natural blend of essential oil which helps prevent from Bacterial/fungal infections, foul smell on the foot, remove suntan, shoe bites etc. on prolonged usage

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## Abstract:

Foot odour is a problem that many people have to deal with on a daily basis. Fungal infection of the feet can cause white and soggy skin between the toes, dry and flaky soles, or reddening and blistering of the skin all over the foot. Around 15% to 25% of people are likely to have athlete's foot at any one time. Foot odour is caused by the metabolic activities of normal bacterial inhabitants of feet. The foot provides a warm, moist environment allowing bacteria such as *Micrococcus* sp., *Staphylococcus* sp., and *Corynebacteria* to thrive. We have used *Citrus medica* (citron oil), *Melaleuca alternifolia* (tea tree oil), *Azadirachta indica* (neem oil) *Mentha piperita* (peppermint oil) and *Eucalyptus* oil along with solvent, fragrance and excipients. *Citrus medica* (Citron) is an underutilized fruit plant having various bioactive components in all parts of the plant. Tea tree oil, an essential oil extracted from the leaves of *Melaleuca alternifolia* by steam distillation and supercritical fluid extraction has found a wide range of antimicrobial activities as antiviral, antifungal, anti-bacterial due to the presence of terpinen-4-ol as the major constituent. *Azadirachta indica* L. (neem) shows therapeutic role in health management due to rich source of various types of ingredients. Leaves contain ingredients such as nimbin, nimbanene, 6-desacetylnimbinene, nimbandiol, nimbolide, ascorbic acid, n-hexacosanol and amino acid, 7-desacetyl-7-benzoylazadiradione, 7-desacetyl-7-benzoylgedunin, 17-hydroxyazadiradione, and nimbiol, Quercetin and  $\beta$ sitosterol, polyphenolic flavonoids, were purified from neem fresh leaves and were known to have antibacterial and antifungal properties. *Mentha piperita* (Lamiaceae family) is one of the herbs most widely used worldwide, with a long history of safe use in medicinal preparations. It is used as antioxidant, antimicrobial, antiviral, anti-inflammatory, and anti-carcinogenic because of its phytochemicals. *Eucalyptus* was the conventional name of *Eucalyptus* of the genus Myrtaceae family. It was native to Australia and was one of the most widely cultivated genera in the world. *Eucalyptus* had been widely used as essential oil from leaves had good biological activities. Essential oils from *Eucalyptus* had been proven to possess strong antibacterial activity and widely applied in the field of medicine, food, and the chemical industry. Further research found that *Eucalyptus* essential oil and its components also exhibited the characteristics of herbicides and insecticides

## Introduction:

The plant kingdom is a rich source of structural biodiversity and offers a variety of natural products. Plants have been utilized to produce various types of medicines for thousands of years. WHO pointed out that more than 80% of world's population depends on plants to meet their primary health care needs. The use of essential oils extracted from plants for clinical purposes have become an important topic in scientific research and industrial application. Thanks to the different biological activities of oils, which exercise antimicrobial, antioxidant, anti-inflammatory, anti – fungal, anti – septic, anti – itch, anti – rash activity. In many countries worldwide aromatic herbs are used in primary health care, especially in rural areas, and 80% of the populations in developing countries use these traditional resources. In this journal we have focused on the different type of natural essential oil which helps to overcome or prevent from foot related diseases. Foot odour is a problem that many people have to deal with on a daily basis. Fungal infection of the feet can cause white and soggy skin between the toes, dry and flaky soles, or reddening and blistering of the skin all over the foot.

Around 15% to 25% of people are likely to have athlete's foot at any one time. Foot odour is caused by the metabolic activities of normal bacterial inhabitants of feet. The foot provides a warm, moist environment allowing bacteria such as *Micrococcus* sp., *Staphylococcus* sp., and *Corynebacteria* to thrive. These particular bacteria use long chain fatty acids released by the sweat and oil glands as food sources. Metabolism of these long chain fatty acids results in the production of shorter volatile fatty acids that are responsible for odours associated with feet. For many individuals this may be a small annoyance, for others, foot odour can be substantial. More seriously, increased growth of these bacteria can provide environmental conditions conducive to infections by dermatophytic fungi. Dermatophytic fungi have the capacity to invade skin cells of the feet causing a more serious condition commonly referred to as athlete's foot or *tinea pedis*.

Etiological agents of dermatophytoses include members in the genera *Epidermophyton*, *Microsporum*, and *Trichophyton*. Dermatophytosis can be very difficult to treat and debilitating for immune compromised individuals infected with these microorganisms. The infection can spread to other parts of the body and to other people. Swimming-pool users and industrial workers may be at increased risk of fungal foot infection. *Citrus medica* oil, *Azadirachta indica* oil, *Mentha piperita* oil, Eucalyptus oil, is used to formulate the foot spray because of its anti – fungal, anti – bacterial, anti – inflammatory, anti – microbial, anti – itch properties.

### **Need of the spray and its purpose:**

We have used *Citrus medica* (citron oil), *Melaleuca alternifolia* (tea tree oil), *Azadirachta indica* (neem oil) *Mentha piperita* (peppermint oil) Eucalyptus oil along with solvent, fragrance and

excipients, because of its extra – ordinary and important physio - chemical properties like anti – inflammatory, anti – oxidant, anti – microbial, anti – fungal, anti – bacterial, anti – septic and anti – itch.

### **The following are the health benefits of the foot spray:**

**Foot sanitizer:** The foot spray has anti-bacterial and antiseptic benefits that helps keep viruses and bacteria away. It sanitizes and moisturizes your feet. Fungal and bacterial infections will disappear due to plants herbal properties and Prevents nail ingrowth caused by fungal and bacterial infections.

**Foot odour:** it keeps the smelly and sweaty feet at bay. Enjoy 24 hours of freshness & fragrance with just 7 to 10 sprays.

**Insect bite:** If you get bitten by mosquitoes, spray on the bite to get immediate relief.

**Crack healing:** The first spray starts the healing process with deep moisturizing, making cracked heels a thing of the past. Regular use helps get rid of Corns & Calluses on the feet.

**Cool foot:** The sensation of burning soles will disappear with the coolness of the foot spray.

**Dead skin:** Roughness due to dead skin will disappear. Say bye to dead skin and hello to soft skin. It has an anti – itch property which gives complete relief from dry, itchy and irritable skin.

**Footwear tan marks:** Get smooth even toned skin with regular use. It helps to remove the foot tan on regular use

**Beneficial in Athlete’s Foot:** A common contagious fungal infection can be treated by using foot spray.

**Diabetic foot:** Heals foot sores that occur due to poor blood circulation.

**Shoe bite:** Helps relief the shoes and footwear bites, Soothes your skin with a couple of sprays.

**Foot protection:** Protects your feet from water cuts, rashes, itchiness etc. caused by rain water. The Foot Spray is great protection against water cuts due to its antiseptic nature.

**Pain reliever:** Walk free, walk tall, with no more heel pain. Regular use of this Foot Spray relieves pain in the heels.

## **Materials and methods:**

***Citrus medica***: Citron oil

## **Taxonomy:**

**Domain:** Eukaryota

**Kingdom:** Plantae

**Phylum:** Spermatophyta

**Subphylum:** Angiospermae

**Class:** Dicotyledonae

**Order:** Rutales

**Family:** Rutaceae

**Genus:** Citrus

**Species:** *Citrus medica*

## **Physio - chemical composition:**

*Citrus medica* (Citron) is an underutilized fruit plant having various bioactive components in all parts of the plant. The major bioactive compounds present are iso-limonene, citral, limonene, phenolics, flavonones, vitamin C, pectin, linalool, decanal, and nonanal, accounting for several health benefits. Pectin and hetero polysachharides also play a major role as dietary fibers. The potential impact of citron and its bioactive components to prevent or reverse destructive deregulated processes responsible for certain diseases has attracted different researchers' attention. The fruit has numerous nutraceutical benefits, proven by pharmacological studies; for example, anti-catarthal, capillary protector, anti-hypertensive, diuretic, antibacterial, antifungal, anthelmintic, antimicrobial, analgesic, strong antioxidant, anti -cancerous, anti-diabetic, estrogenic, antiulcer, cardio protective, and anti-hyperglycemic. In the article, the fruit taxonomical classification, beneficial phytochemicals, antioxidant activities, are discussed.

The fruit peel oil contains iso-limonene (39.37%) citral (23.12%) and limonene (21.78%) as major components and the fruit is rich in vitamins and minerals, particularly vitamin A, vitamin C, niacin and thiamine. The fruit peel essential oils have several phytochemicals with high free radical scavenging and anti-fungal activity. The fruit-pulp has numerous nutraceutical importance, it is an anti-catarrrhal, capillary protector, anti-hypertensive, diuretic, antibacterial, antifungal, anti-helminthic, antimicrobial, analgesic, strong antioxidant, anti-cancerous, anti-diabetic, estrogenic, antiulcer, cardioprotective, antihyperglycemic etc.

### **Antimicrobial activity:**

Antimicrobial agents inhibit microorganism's growth with the help of enzymes that hinders the essential pathways of the microbes; block their physiological, metabolic activities as well as reproduction. Plant based antimicrobials have enormous therapeutic potential as they can serve the purpose with lesser side effects that are often associated with synthetic antimicrobials. Continued further exploration of plant-derived antimicrobials is needed today. It was found that the extract of peels was effective against *Staphylococcus aureus*, *Proteus vulgaris*, *Klebsiella pneumonia*, *Eschheria coli*, *Bacillus subtilis* and *Pseudomonas aeruginosa*. Further research is necessary to determine the identity of the antibacterial compounds from the peels of *Citrus medica* L. and also to determine their full spectrum of efficacy. *Citrus medica* L. peel extract possess a broad spectrum of activity against a panel of bacteria responsible for the most common bacterial diseases. This extract opens the possibility of finding new clinically effective antibacterial compounds

### **Antioxidant activity**

*C. medica* tends to possess a huge amount of antioxidants in the peel as well as pulp. The free radicals are typically highly reactive short-lived species with an unpaired valence electron and causes oxidative damage, oxidative stress and mutations in the genes, thus leading to the development of oncogenes, initiators of the malignant tumours. The antioxidants have an immense effect in prevention of cancer. Antioxidants controls uncontrolled mitosis and thus can aids on the cancer patients in their treatment. studied the antioxidant and free radical scavenging activity of *C. medica* extracts and the antioxidant activity was determined by total phenol content, DPPH and Nitric oxide radical scavenging effect. The phytochemical investigation showed the presence of alkaloid, carbohydrate, Glycoside, Triterpenoids, Resins and Tannins which work as antioxidants. According to different studies the highest antioxidant activity is observed in the peel extract by reductive ability method. Phenolic compound and ascorbic acid are identified as possible antioxidants in citron. Phenolic compound were able to scavenge radicals and to chelate metals while ascorbic acid can play a pro-oxidant role in the presence of transition metals.

The *C. medica* extracts can effectively scavenge various free radicals under in vitro conditions. The antioxidant activity is due to the presence of phenolic compound or vitamin C present in the fruits

### Anti hyperglycemic activity:

*C. medica* can be used as a natural product for management of diabetes foot disease.

### Test and results:

The physical analysis of the *Citrus medica* oil has shown the following properties:

### Result:

<b>Product name</b>		Citron oil		
<b>Source</b>		<i>Citrus medica</i>		
<b>Parts used</b>		Citron peel		
<b>Test parameters</b>		<b>Result</b>		
Method of extraction		Cold pressed		
Colour and appearance @22°C		Pale yellow to greenish yellow		
Lot number		CB/CMO/FS		
Odour		Strong bright lemony odor		
Flash point		49°C		
Solubility		Soluble in alcohol, insoluble in water		
<b>Physio – chemical properties</b>	<b>Specification</b>	<b>Batch No: CBCM001</b>	<b>Batch No: CBCM002</b>	<b>Batch No: CBCM003</b>
Specific Gravity @20°C(g/ml)	0.865 – 0.890	0.874	0.881	0.869
Optical Rotation @ 20°C(Degrees)	+60 to +70	+67	+63	+67
Refractive index @ 20°C	1.472 to 1.475	1.474	1.473	1.474
<b>Microbial test</b>	<b>Specification</b>	<b>Batch No: CBCM001</b>	<b>Batch No: CBCM002</b>	<b>Batch No: CBCM003</b>
Aerobic total plate count	<10000CFU/ml	<10CFU/ml	<10CFU/ml	<10CFU/ml
Yeast and mold	<100 CFU/ml	<10CFU/ml	<10CFU/ml	<10CFU/ml
E. coli	Negative	Negative	Negative	Negative
Salmonella	Negative	Negative	Negative	Negative
Staphylococcus sp	Negative	Negative	Negative	Negative
<b>Shelf life</b>	24 Months under specific storage condition			

<b>Storage condition</b>	Store in well closed container away from moisture, freezing and direct heat
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## **Azadirachta indica: Neem oil**

### **Taxonomy:**

**Domain:** Eukaryota

**Kingdom:** Plantae

**Phylum:** Spermatophyta

**Subphylum:** Angiospermae

**Class:** Dicotyledonae

**Order:** Rutales

**Family:** Meliaceae

**Genus:** Azadirachta

**Species:** *Azadirachta indica*

### **Physio – chemical composition.**

Neem has become valuable plant in the world which shows the solutions for hundreds to thousands problems. Neem (*Azadirachta indica*) commonly called ‘Indian Lilac’ or ‘Margosa’. Neem has been extensively used in Ayurveda, Unani and Homoeopathic medicine and has become a centre of attraction of modern medicine. The important pharmacological activities like anti-inflammatory, antimalarial, anti-bacterial, anti-allergic, anti - dermatic, antiulcer, antifungal, insecticidal, larvicidal and other pharmacological activities.

*Azadirachta indica* L. (neem) shows most important active constituent is azadirachtin and the others are nimbolinin, nimbin, nimbidin, nimbidol, sodium nimbinat, gedunin, salannin, and quercetin. Leaves contain ingredients such as nimbin, nimbanene, 6-desacetylnimbinene, nimbandiol, nimbolide, ascorbic acid, n-hexacosanol and amino acid, 7-desacetyl-7- benzoylazadiradione, 7-desacetyl-7-benzoylgedunin, 17-hydroxyazadiradione, and nimbiol, Quercetin and  $\beta$ sitosterol, polyphenolic

flavonoids, were purified from neem fresh leaves and were known to have antibacterial and antifungal properties.

### Anti – fungal activity

Neem extract is very active against skin fungi which cause the ringworm disease. addition to antifungal activity, presence of high concentrations of azadirachtins, quercetin and  $\beta$ -sitosterol in A. Indica leaves might be responsible for strong antibacterial and anti-fungal activity . Leaf extract exhibited strong antimicrobial activity against bacteria and fungi. Cyclic trisulphide and cyclic tetrasulphide exhibits Antifungal activity

### Anti – inflammatory activity:

A study result has confirmed that extract of A. indica leaves showed significant anti-inflammatory activity. It suggest that nimbidin suppresses the functions of macrophages and neutrophils relevant to inflammation. neem oil showed significant anti- inflammatory effect in both acute as well as chronic inflammation, it was also found to have low ulcerogenic potential compared to Indomethacin, hence can be safely used as a potent anti-inflammatory agent.

### Anti- microbial activity:

Oil extracted from leaves gives a wide spectrum of antibacterial activity action against gram positive and gram negative microorganisms which including M. tuberculosis and streptomycin resistant strains. . Azadirachta indica shows antibacterial activity against two different bacterial strains i.e. E. coli and B. subtilus. The photoconstituents like alkaloids, steroids, tennis, crude glycosides and flavonoids of neem plants was tested for antibacterial activity against pathogenic strains of E. coli, Corynebacterium bovis and Staphylococcus aureus. The outcomes reported that flavonoids compounds have antimicrobial activity crude saponins prevent the growth of the microbes

### Test and results:

The physical analysis of the *Azadirachta indica* oil has shown the following properties:

#### Result:

<b>Product name</b>	Neem leaf oil
<b>Source</b>	<i>Azadirachta indica</i>
<b>Parts used</b>	Leaves
<b>Test parameters</b>	<b>Result</b>



Method of extraction		Cold pressed		
Colour and appearance @22°C		Yellowish green to dark brown liquid		
Lot number		CB/AIO/FS		
Odour		Characteristic of Neem fragrant odor		
Flash point		400°C		
Solubility		Soluble in alcohol, insoluble in water		
<b>Physio – chemical properties</b>	<b>Specification</b>	<b>Batch No: CBAI001</b>	<b>Batch No: CBAI002</b>	<b>Batch No: CBAI003</b>
Specific Gravity @20°C(g/ml)	0.918 to 0.965	0.925	0.954	0.927
Optical Rotation @ 20°C(Degrees)	-20° to -47°	-35°	-30°	-43°
Refractive index @ 20°C	1.450 to 1.460	1.455	1.456	1.455
<b>Microbial test</b>	<b>Specification</b>	<b>Batch No: CBAI001</b>	<b>Batch No: CBAI002</b>	<b>Batch No: CBAI003</b>
Aerobic total plate count	<10000CFU/ml	<10CFU/ml	<10CFU/ml	<10CFU/ml
Yeast and mold	<100 CFU/ml	<10CFU/ml	<10CFU/ml	<10CFU/ml
E. coli	Negative	Negative	Negative	Negative
Salmonella	Negative	Negative	Negative	Negative
Staphylococcus sp	Negative	Negative	Negative	Negative
<b>Shelf life</b>	24 Months under specific storage condition			
<b>Storage condition</b>	Store in well closed container away from moisture, freezing and direct heat			

## Mentha piperita: Peppermint oil

### Taxonomy:

**Domain:** Eukaryota

**Kingdom:** Plantae

**Phylum:** Spermatophyta

**Subphylum:** Angiospermae

**Class:** Dicotyledonae

**Order:** Lamiales

**Family:** Lamiaceae

**Genus:** Mentha

**Species:** *Mentha piperita*

## Physio chemical composition:

Among the diversity of plants, *Mentha piperita* (Lamiaceae family) is one of the herbs most widely used worldwide, with a long history of safe use in medicinal preparations. It is used as antioxidant, antimicrobial, antiviral, anti - inflammatory, and anti-carcinogenic. Plant is known for having several phytochemicals, including polyphenols that are highly effective antioxidants and are less toxic than the synthetic ones. Some of the beneficial biological effects show that this plant may play an important role as anti-oxidant, antinociceptive, anti- inflammatory, antimicrobial, anti-carcinogenic, antiviral, anti-allergic and anti - tumorigenic, indicating its utility in the prevention or treat several disease.

In the extract of the leaves of *M. piperita* are present mainly flavonoids and phenolic acids and some of the compounds are menthol, menthone caffeic acid, acetaldehyde, amyl alcohol, menthyl esters, limonene, pinene, cardial glycosides, phellandrene, cadinene, pugelone, and dimethyl sulfide. The constituent features include alpha-pinene, sabinene, terpinolene, ocimene, diterpenes, gamma-terpinene, steroids, fenchene, alpha- and beta-thujone, coumarin, citronellol, carotenes, tocopherols, betaine, choline, saponin, tannins, and other components. The presence of flavonoids such as eriocitrin, narirutin, hesperidin, luteolin-7-O-rutinoside, isorhoifolin, diosmin, rosmarinic acid, and 5, 7-dihydroxycromone-7- O-rutinoside exert anti-allergic effects.

### Anti – oxidant activity

*Mentha pipertita* have antioxidant properties due to presence of several bioactive substances.

### Anti – microbial activity

Menthol and menthone present in the essential oil components of *M. piperita* is responsible for the antimicrobial activity.

### Test and results:

The physical analysis of the *Mentha piperita* oil has shown the following properties:

#### Result:

<b>Product name</b>	Peppermint oil
<b>Source</b>	<i>Mentha piperita</i>
<b>Parts used</b>	Leaves
<b>Test parameters</b>	<b>Result</b>

Method of extraction		Steam Distillation		
Colour and appearance @20°C		Colourless to pale greenish - yellow		
Lot number		CB/MPO/FS		
Odour		Characteristic peppermint odour		
Flash point		163°F		
Solubility		Soluble in alcohol. Insoluble in water		
<b>Physio – chemical properties</b>	<b>Specification</b>	<b>Batch No: CBMP001</b>	<b>Batch No: CBMP002</b>	<b>Batch No: CBMP003</b>
Specific Gravity @20°C(g/ml)	0.890 to 0.910	0.915	0.905	0.892
Optical Rotation @ 20°C(Degrees)	-30° to -34°	-33°	-30°	-32°
Refractive index @ 20°C	1.459 – 1.465	1.462	1.463	1.462
<b>Microbial test</b>	<b>Specification</b>	<b>Batch No: CBMP001</b>	<b>Batch No: CBMP002</b>	<b>Batch No: CBMP003</b>
Aerobic total plate count	<10000CFU/ml	<10CFU/ml	<10CFU/ml	<10CFU/ml
Yeast and mold	<100 CFU/ml	<10CFU/ml	<10CFU/ml	<10CFU/ml
E. coli	Negative	Negative	Negative	Negative
Salmonella	Negative	Negative	Negative	Negative
Staphylococcus sp	Negative	Negative	Negative	Negative
<b>Shelf life</b>	24 Months under specific storage condition			
<b>Storage condition</b>	Store in well closed container away from moisture, freezing and direct heat			

## Eucalyptus: Eucalyptus oil

### Taxonomy:

**Domain:** Eukaryota

**Kingdom:** Plantae

**Phylum:** Spermatophyta

**Subphylum:** Angiospermae

**Class:** Dicotyledonae

**Order:** Myrtales

**Family:** Myrtaceae

**Genus:** *Eucalyptus*

## **Physio – chemical properties:**

Eucalyptus was the conventional name of Eucalyptus of the genus Myrtaceae family. Eucalyptus had been widely used in plywood, pulp, and solid wood production, as well its essential oil from leaves had good biological activities. Essential oils from eucalyptus had been proven to possess strong antibacterial activity and widely applied in the field of medicine, food, and the chemical industry. The oil composition is identified and main components in essential oils from eucalyptus species are presented in increasing order of 1,8-cineole content.

The main components are monoterpenes (1,8-cineole, p-cymene, citronellal, citronellol, limonene,  $\alpha$ -phellandrene,  $\beta$ -phellandrene,  $\alpha$ -pinene,  $\beta$ -pinene, trans-pinocarveol, terpinolene,  $\alpha$ -terpineol,  $\alpha$ -thujene) and sesquiterpenes ( $\beta$ -caryophyllene,  $\beta$ -eudesmol, globulol, spathulenol and viridiflorol). The chemical profile and main components of oils from eucalyptus leaves varied significantly between species. For example, the main components of *E. largiflorens* oil were 1,8-cineole (37.5%), p-cymene (17.4%), neo-isoverbenol (9.1%), limonene (6.5%) and spathulenol (6.7%), while those of *E. spathulata* oil were 1,8-cineole (72.5%),  $\alpha$ -pinene (12.7%) and trans-pinocarveol (3.3%), with the absence of neo-isoverbenol, limonene and spathulenol or at lower amounts (88). The monoterpenes, 1,8-cineole and  $\alpha$ -pinene, are the main components in most species, while, *E. citriodora* is rich in citronellal (49.5-87%) and citronellol (8-20%). The content of 1,8-cineole in eucalyptus oils ranges from 10-90%. Generally, the content of  $\alpha$ -pinene is below 20%. The composition of eucalyptus oils differs even between the trees of same species in different periods, sites and extraction method/time

## **Anti - microbial activity:**

Eucalyptus essential oils had antibacterial activity against six bacteria (*Staphylococcus aureus*, *Bacillus cereus*, *Escherichia coli*, *Micrococcus luteus*, *Proteus mirabilis* and *Alcaligenes faecalis*). It is showed that *Haemophilus influenzae*, *Haemophilus parainfluenzae* and *Stenotrophomonas maltophilia* were the most susceptible to *E. globulus* essential oils, followed by *Streptococcus pneumoniae* and *Streptococcus agalactiae*. The highest activity was found at 1.25  $\mu$ l ml<sup>-1</sup> for *H. influenzae*, *H. parainfluenzae* and *S. maltophilia*.

## **Anti – viral activity:**

Antiviral activity assays using virus yield experiments indicated mild activity on the mumps virus.

## **Anti - fungal activity:**

E. globulus oils are antibacterial and antifungal. It was more effective than prevalent synthetic antifungal drugs (Dactrine, Nizral and Tenaderm) without any adverse side effects on mammalian skin up to 5% concentration.

### Anti - oxidant activity:

Eucalyptus oils also antioxidant, due to the presence of phenolic compounds and their radical scavenging properties. They can also trigger a series of induced chemical defence responses.

### Test and results:

The physical analysis of the **Eucalyptus oil** has shown the following properties:

### Result:

<b>Product name</b>		<b>Eucalyptus oil</b>		
<b>Source</b>		Eucalyptus globulus		
<b>Parts used</b>		Leaves		
<b>Test parameters</b>		<b>Result</b>		
Method of extraction		Steam distillation		
Colour and appearance @20°C		Colourless to pale yellow		
Lot number		CB/EO/FS		
Odour		Characteristic herbal odor		
Flash point		99°C		
Solubility		Soluble in other oils. Insoluble in water		
<b>Physio – chemical properties</b>	<b>Specification</b>	<b>Batch No: CBEO001</b>	<b>Batch No: CBEO002</b>	<b>Batch No: CBEO003</b>
Specific Gravity @20°C(g/ml)	0.897 to 0.924	0.915	0.919	0.922
Optical Rotation @ 20°C(Degrees)	+2 to +9	+5	+5	+4
Refractive index @ 20°C	1.457 – 1.469	1.462	1.459	1.466
<b>Microbial test</b>	<b>Specification</b>	<b>Batch No: CBEO001</b>	<b>Batch No: CBEO002</b>	<b>Batch No: CBEO003</b>
Aerobic total plate count	<10000CFU/ml	<10CFU/ml	<10CFU/ml	<10CFU/ml
Yeast and mold	<100 CFU/ml	<10CFU/ml	<10CFU/ml	<10CFU/ml
E. coli	Negative	Negative	Negative	Negative
Salmonella	Negative	Negative	Negative	Negative
Staphylococcus sp	Negative	Negative	Negative	Negative
<b>Shelf life</b>	24 Months under specific storage condition			

<b>Storage condition</b>	Store in well closed container away from moisture, freezing and direct heat
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## **Melaleuca alternifolia: tea tree oil**

### **Taxonomy:**

**Kingdom:** Plantae

**Clade:** Tracheophytes

**Clade:** Angiosperms

**Clade:** Eudicots

**Clade:** Rosids

**Order:** Myrtales

**Family:** Myrtaceae

**Genus:** Melaleuca

**Species:** Melaleuca alternifolia

### **Physio chemical properties:**

Tea tree oil is the essential oil obtained from Melaleuca alternifolia, Melaleuca linariifolia and Melaleuca dissitiflora as well as other species of Melaleuca. Tea Tree Oil from Melaleuca alternifolia contains various mono- and sesquiterpenes as well as aromatic compounds. In World War II, TTO was utilized as an insect repellent and a general anti-microbial agent. Later, its applications have expanded, and its popularity in various industries is currently soaring as it is used such as a preservative, fungicide, natural biocide and in cosmetics, aromatherapy, allopathic and herbal medicines, etc. TTO is transparent to slightly yellow in colour, with a cooling effect similar to that of menthol and a strong odour just like camphor

The report states that tea tree oil consist of approximately 100 components of different concentrations whose composition are regulated by an international Organization for Standardization standard as (ISO4730) It consist of terpinen-4-ol,  $\gamma$ -terpinene,  $\alpha$ -terpinene, 1,8-Cineole,  $\alpha$ -terpinolene  $\alpha$ -terpineol  $\alpha$ -pinene, p-Cymene, The monoterpenes terpinen-4-ol,  $\alpha$ -terpinene, 1,8-cineole, p-cymene, terpinolenes, limonene and sabinene account for 80 - 90% of the oil. I-beta-Pinene  $\beta$ -Pinene  $\alpha$ -Terpinene, Eucalyptol,  $\alpha$ -Gurjunene (+)-Gurjunene - Aromadendrene ,  $\delta$ -Cardinene,  $\beta$  -Gurjunene is also present in TTO.

### **Anti – microbial activity:**

The major components terpinen-4-ol and 1,8-cineole are the main antimicrobial agents. This oil has been used as a strong antifungal and antimicrobial agent in soaps, creams, toothpastes, etc. It has also been examined for its effects in various superficial infections such as tinea, acne, oral candidiasis, and cold sores. The prime component of the oil, i.e., terpinen-4-ol, exerted antimicrobial effect against all microbes tested, while alpha pinene and linalool were successful against *Bacillus cereus* and *Bacillus subtilis*

### **Anti- bacterial activity**

*M. alternifolia* oil has bactericidal activity against *E. coli*, *Staphylococcus* species, *Lactobacillus*, and *Actinomyces viscosus*

### **Anti – fungal activity**

The effective fungicidal capability of tea tree oil against *Candida*, *Aspergillus*, and *Trichophyton* species. TTO was analyzed in an experiment consisting in treating invasive fungal wound infections (IFIs) using natural medicine. The results indicated that the oil was very effective as it inhibited the growth of filamentous fungi causing IFIs. It is also reported that *M. alternifolia* has anti- fungal properties against *Aspergillus flavus*.

### **Anti – oxidant activity**

*M. alternifolia* oil has promising antioxidant abilities.  $\alpha$ -terpinene,  $\alpha$ -terpinolene, and  $\gamma$ -terpinene have higher anti-oxidative

### **Anti – septic activity**

The volatile oil from *Melaleuca alternifolia* species at a 5% concentration had also passed the Therapeutic Goods Act test for antiseptics and disinfectants.

### **Test and results:**

The physical analysis of the *Melaleuca alternifolia* (tea tree oil) has shown the following properties:

#### **Result:**

<b>Product name</b>	Tea tree oil
<b>Source</b>	<i>Melaleuca alternifolia</i>
<b>Parts used</b>	Leaves
<b>Test parameters</b>	<b>Result</b>
Method of extraction	Steam Distillation

Colour and appearance @20°C		Liquid colourless to pale yellow		
Lot number		CB/MAO/FS		
Odour		Characteristic		
Flash point		55°C		
Solubility		Insoluble in water, soluble in alcohol		
<b>Physio – chemical properties</b>	<b>Specification</b>	<b>Batch No: CBMA001</b>	<b>Batch No: CBMA002</b>	<b>Batch No: CBMA003</b>
Specific Gravity @20°C(g/ml)	0.880 to 0.904	0.887	0.883	0.901
Optical Rotation @ 20°C(Degrees)	+6° to +10°	+9.57	+7°	+6°
Refractive index @ 20°C	1.476 to 1.482	1.4772	1.478	1.481
<b>Microbial test</b>	<b>Specification</b>	<b>Batch No: CBMA001</b>	<b>Batch No: CBMA002</b>	<b>Batch No: CBMA003</b>
Aerobic total plate count	<10000CFU/ml	<10CFU/ml	<10CFU/ml	<10CFU/ml
Yeast and mold	<100 CFU/ml	<10CFU/ml	<10CFU/ml	<10CFU/ml
E. coli	Negative	Negative	Negative	Negative
Salmonella	Negative	Negative	Negative	Negative
Staphylococcus sp	Negative	Negative	Negative	Negative
<b>Shelf life</b>	24 Months under specific storage condition			
<b>Storage condition</b>	Store in well closed container away from moisture, freezing and direct heat			

## Extraction procedure for the Essential oil

### STEAM DISTILLATION

Steam Distillation is the most popular method used to extract and isolate essential oils from plants for use in natural products. This happens when the steam vaporizes the plant material's volatile compounds, which eventually go through a condensation and collection process. Many old-time distillers favor this method for most oils, and say that none of the newer methods produces better quality oils. Steam distillation is a special type of distillation or a separation process for temperature sensitive materials like oils, resins, hydrocarbons, etc. which are insoluble in water and may decompose at their boiling point. The fundamental nature of steam distillation is that it enables a compound or mixture of compounds to be distilled at a temperature substantially below that of the boiling point(s) of the individual constituent(s).



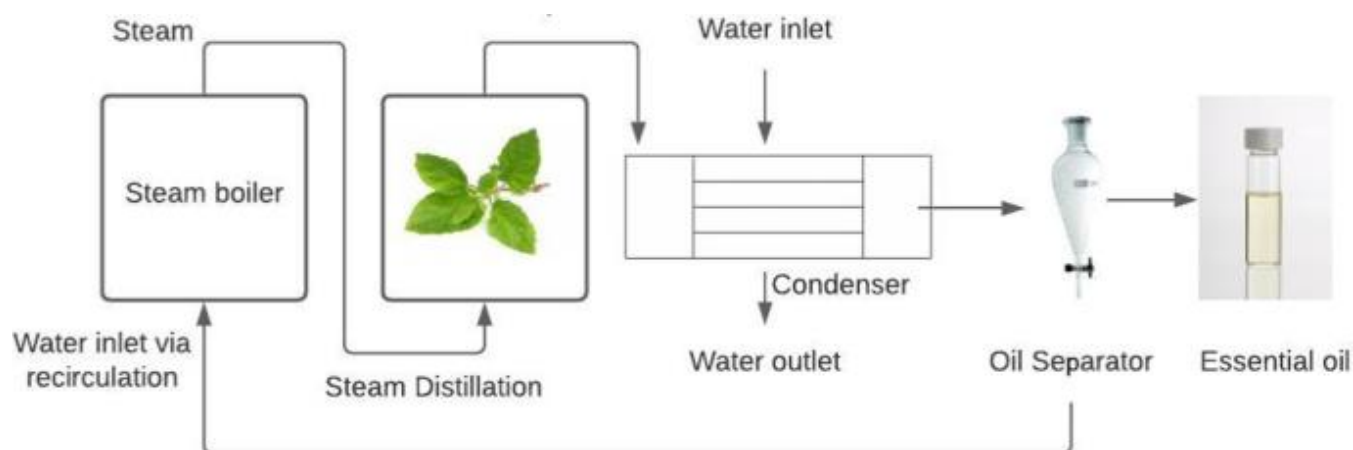
Essential oils contain substances with boiling points up to 200°C or higher temperatures. In the presence of steam or boiling water, however, these substances are volatilized at a temperature close to 100°C at atmospheric pressure. Fresh, or sometimes dried, botanical material is placed in the plant chamber of the still and the steam is allowed to pass through the herb material under pressure which softens the cells and allows the essential oil to escape in vapour form. The temperature of the steam must be high enough to vaporize the oil present, yet not so high that it destroys the plants or burns the essential oils. As they are released, the tiny droplets of essential oil evaporate and, together with the steam molecules, travel through a tube into the still's condensation chamber. As the steam cools, it condenses into water. The essential oil forms a film on the surface of the water. To separate the essential oil from the water, the film is then decanted or skimmed off the top. The remaining water, a byproduct of distillation, is called floral water, distillate, or hydrosol. It retains many of the therapeutic properties of the plant, making it valuable in skin care for facial mists and toners. In certain situations, floral water may be preferable to be pure essential oil, such as when treating a sensitive individual or a child, or when a more diluted treatment is required.

Essential oil isolated by steam distillation are different in composition to those naturally occurring in the oil bearing glands of plants, since the steam distillation conditions cause chemical reactions to occur which result in the formation of certain artificial chemicals, called artifacts. Some of these are considered beneficial. Few, if any, essential oils are unscathed by the thermal conditions of steam distillation, but some distillation techniques can, in certain instances, be a measure less damaging than others (e.g. hydro diffusion – a sort of inverted steam distillation where steam is introduced at the top of the vegetable material-packed container, and oil and condensate issue from the bottom – can produce oils with higher ester contents i.e. less thermally induced hydrolysis). A number of factors determine the final quality of a steam distilled essential oil.

Aside from the plant material itself, most important are time, temperature and pressure, and the quality of the distillation equipment. Essential oils are very complex products. Each is made up of many, sometimes hundreds, of distinct molecules which come together to form the oil's aroma and therapeutic properties. Some of these molecules are fairly delicate structures which can be altered or destroyed by adverse environmental conditions. So, much like a fine meal is more flavorful when made with patience, most oils benefit from a long, slow 'cooking' process. It is possible that longer distillation times may give more complete oil. It is also possible however, that longer distillation time may lead to the accumulation of more artifacts than normal. This may have a curious effect of appearing to improve the odour, as sometimes when materials that have a larger number of components are sniffed, the perception is often of slightly increased sophistication, added fullness and character, and possibly, and extra pleasantness

## STEAM DISTILLATION PROCESS

- A large container called a *Still*, which is usually made of stainless steel, containing the plant material has steam added to it.
- Through an inlet, steam is injected through the plant material containing the desired oils, releasing the plant's aromatic molecules and turning them into vapour.
- The vaporized plant compounds travel to the condensation flask or the *Condenser*. Here, two separate pipes make it possible for hot water to exit and for cold water to enter the Condenser. This makes the vapour cool back into liquid form.
- The aromatic liquid by-product drops from the Condenser and collects inside a receptacle underneath it, which is called a *Separator*. Because water and oil do not mix, the essential oil floats on top of the water. From here, it is siphoned off. (*Some essential oils are heavier than water, such as clove essential oil, so they are found at the bottom of the Separator.*)



### Formulation of foot spray:

We have used the following essential oil to formulate the foot spray along with fragrance, excipients and solvents.



*Melaleuca alternifolia* oil



*Citrus medica* oil



*Eucalyptus* oil



*Mentha piperita* oil



*Azadirachta indica* oil

**Qualitative and quantitative composition:**

S.No	Name of the ingredients	%
1	Melaleuca alternifolia oil	5
2	Citrus medica oil	5
3	Azardirachta indica oil	2
4	Mentha piperita oil	2
5	Eucalyptus oil	2
6	Fragrance	Lavender, morning glory, citrus
7	Excipient	Glycerine
8	Solvent	Iso propyl alcohol



**Foot spray**

**Physio chemical composition:**

We have used the essential oils like Melaleuca alternifolia (tea tree oil) 5%, Citrus medica (peel) oil 5%, Azardirachta indica (leaf) oil 2%, Mentha piperita oil 2%, Eucalyptus oil 2%, , along with fragrance, Excipients and solvent to formulate this foot spray.

It consists of iso-limonene, citral, limonene, phenolics, flavonones, vitamin C, pectin, linalool, Quercetin and  $\beta$ -sitosterol, polyphenolic flavonoids, eriocitrin, narirutin, hesperidin, luteolin-7-O-rutinoside, isorhoifolin, diosmin, rosmarinic acid, and 5, 7-dihydroxycromone-7- O-rutinoside, monoterpenes (1,8-cineole, p-cymene, citronellal, citronellol, limonene,  $\alpha$ -phellandrene,  $\beta$ phellandrene,  $\alpha$ -pinene,  $\beta$ -pinene, trans-pinocarveol, terpinolene,  $\alpha$ -terpineol,  $\alpha$ -thujene) and sesquiterpenes, I-beta-Pinene  $\beta$ -Pinene  $\alpha$ -Terpinene, Eucalyptol,  $\alpha$ -Gurjunene, (+)-Gurjunene - Aromadendrene ,  $\delta$ -Cardinene,  $\beta$  –Gurjunene.

## Test and results:

The physical analysis of the **Foot spray** has shown the following properties:

### Result:

Test parameters		Result		
Colour and appearance @20°C		Colourless to Pale yellow		
Lot number		CB/FS/001		
Odour		Mixed herb smell		
Flash point		400°C		
Solubility		Insoluble in water, soluble in alcohol		
Physio – chemical properties	Specification	Batch 1	Batch 2	Batch 3
Specific Gravity @20°C(g/ml)	0.870-0.910	0.877	0.909	0.089
Microbial test	Specification	Batch 1	Batch 2	Batch 3
Aerobic total plate count	<10000CFU/ml	<10CFU/ml	<10CFU/ml	<10CFU/ml
Yeast and mold	<100 CFU/ml	<10CFU/ml	<10CFU/ml	<10CFU/ml
E. coli	Negative	Negative	Negative	Negative
Salmonella	Negative	Negative	Negative	Negative
Staphylococcus sp	Negative	Negative	Negative	Negative
<b>Shelf life</b>	24 Months under specific storage condition			
<b>Storage condition</b>	Store in well closed container away from moisture, freezing and direct heat			

### Uses:

It is responsible for the anti – inflammatory, anti – oxidant, anti – septic, anti – itch, anti – water cut, anti – fungal & anti – bacterial properties. It is also beneficial for diabetic, athlete's foot and other foot related disease. It helps prevent from Eczema, Itching, Rashes, Fungal infection, Tan mark, Ring worms, Dead skin, hard and sore cracked heels, Blisters, Burnings, dry skin.

### **Therapeutic indication:**

1. Heals and softens cracked heels.
2. Cools and relaxes tired feet.
3. Removes dead skin.
4. Helps in Removal of Footwear Tan Marks on feet.
5. This foot spray acts as a foot sanitizer which has Anti-Bacterial, Antifungal and Antiseptic benefits that helps keep viruses and bacteria away.
6. It sanitizes and moisturizes your feet.
7. Can be used in all seasons
8. Non greasy, Non Slippery
9. No more foul smelling foot
10. Helps from White, soggy skin in between your toes, which may tear to produce fissures.
11. Free from gas
12. Can be used by all ages (Except Infants)
13. Helps relieve the shoes and footwear bites, Soothes your skin with a couple of sprays.
14. It helps in contagious fungal infection athlete's foot
15. It helps relieve foot sores that occur due to poor blood circulation
16. This can be used by pregnant and lactating mothers
17. No harm human health

### **Caution:**

1. Keep in cool place away from direct sunlight.
2. Do not expose to temperature exceeding 50° C.
3. Do not spray near open flame (Extremely Flammable).
4. Avoid contact with eyes. If contact occurs, wash with water and consult a doctor.
5. Keep out of reach from children.
6. If you get any allergic reaction stop using and consult a physician
7. Not for infants.

## **Posology and method of administration:**

1. Hold the bottle straight and spray
2. Keep the distance of 5cm away from the foot and spray
3. Shake well before use
4. For better result 7 sprays per foot each day
5. Can use more than 7 sprays as per your need
6. Can spray even on socks
7. Its unisex foot spray

## **Warning:**

1. Do not buy if the seal is opened or broken
2. Don't spray on open cuts and wounds
3. Do patch test before use
4. If u r allergic, avoid usage and seek physician advise

## **Conclusion:**

Nature is our best friend which provides us all the resources to live here. It gives us water to drink, pure air to breathe, food to eat, land to stay, animals, plants for our other uses, etc for our betterment. One such natural product from plant is essential oil. Essential oil has gained much fame and become an integral part of the pharmaceutical, agriculture, food, and cosmetic industries in the world. Its importance lies in its complex composition which is characterized by high levels of antimicrobial, antioxidant, antifungal, anti-inflammatory, anti -diabetic, anti – septic, anti-allergic properties.

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