



WORLD JOURNAL OF CURRENT MEDICAL AND PHARMACEUTICAL RESEARCH

www.wjcmpr.com

ISSN: 2582-0222

A total survey on leaves of *Melaleuca alternifolia* (tea tree oil)

Sankara Malathi* Sidde Lahari, Konapalli Rajani, Mahalakshmi Sampagavi, M. Sushma.

Assistant professor, Department of Pharmaceutical Chemistry, KR College of Pharmacy, Bangalore, India.

ABSTRACT

Tea tree oil, a basic oil extricated from the leaves of *Melaleuca alternifolia* by steam refining, supercritical liquid extraction, soxhlet extraction followed by progressive extraction and small scale stove extraction forms by various solvents has discovered a wide scope of antimicrobial exercises as antiviral, antifungal, antibacterial, against protozoal because of the nearness of terpinen-4-ol as the significant constituent. TTO was contain flavonoids, glycosides, quinolines, starches and so forth. TTO is considered and the rate yield of tea tree oil extricated is resolved and the piece of tea tree absolutes (terpinen-4-ol, 1,8-cineole, γ -terpinene and α -terpineol) separated was contrasted and standard tea tree oil just as with ISO 4730 TTO has discovered the mitigating and hostile to skin break out vulgaris, Psoriasis, free radical rummaging exercises. Tea tree oil is normal items, so it is non-harmful, effectively available, biodegradable, and biocompatible. The few points of interest of tea tree oil make it one of the gainful item having helpful impacts. The current audit article depends on the use of tea tree oil, extraction procedure of tea tree oil, constituents, security contemplations and so on.

Key words:

Tree tea oil, *Melaleuca alternifolia*, Extraction methods, phytochemical analysis, chemical composition, pharmacological activity.

Article History:

Received On: 14.06.2020

Revised On: 05.08.2020

Accepted On: 09.08.2020

*Corresponding Author

Name: Sankara Malathi

Email: sankaramalathi94@gmail.com

DOI: <https://doi.org/10.37022/wjcmpr.vi.152>

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INTRODUCTION

Plants have for some time been perceived as an important wellspring of restorative operators. Specifically, optional plant metabolites, for example, fundamental oils have been utilized since the beginning for helpful purposes [1]. Numerous corresponding and elective prescriptions have delighted in expanded prevalence in ongoing decades. Endeavors to approve their utilization have seen their putative helpful properties go under expanding investigation in vitro and, at times, in vivo. One such item is tea tree oil (TTO), the unpredictable basic oil got chiefly from the Australian local plant *Melaleuca alternifolia* [2]. The basic oil that is steam refined from the Australian local plant *Melaleuca alternifolia* (Myrtaceae), otherwise called melaleuca oil or tea tree oil (TTO), Tea tree oil is fundamental oil which is extricated from the leaves of the *Melaleuca alternifolia*. It has a camphoraceous smell and shading that ranges from light yellow to almost dry [3]. Australian tea tree oil is a famous therapeutic oil got from the subepidermal oil organs in leaves of *Melaleuca alternifolia* (Lady and Bette) Cheel by steam refining or hydrodistillation (Southwell and Lowe, 1999) [4]. *Melaleuca alternifolia* sp. or then again usually known as the Australian tea tree plant, had been highly known for their conventional helpful and therapeutic properties, got from the sub-epidermal oil organs, for the most part from their leaves pores [5]. Most essential oils available today are extracted by steam distillation among, although there are arious extraction methods such as distillation, CO₂ supercritical extraction, and solvent extraction [6] followed by successive extraction [7]. It's the oldest form of

essential oil extraction, quite simple and the best method for distilling leafy materials. Moreover, this process not only causes minimum changes to the essential oil composition during extraction, but also the steam is readily available, cheap, not hazardous and can be recycled. So steam distillation is used to collect crude TTO (35 – 45% of volume of Terpinen-4-ol) [8]. After steam distillation, the commercial value of TTO is not high, so it should be refined to increase its commercial value and fit with tea tree oil standard. Some refinement methods are vacuum distillation, crystallization, column chromatography [8].

A microwave-helped dry strategy for removing basic oils was portrayed by Craveiro et al. (1989). The oil delivered from *Lippia sidoides* was subjectively like the steam refined oil yet essentially unique quantitatively. Firm (1995) and Southwell et al. (1995) have utilized microwave-helped dissolvable extraction for the fast GC examination of tea tree leaf tests down to 1 mg. They found that 10 s of microwaving decreased extraction time for tea tree leaf from 30 h to 1 h. They likewise analyzed the potential for a microwave pretreatment to liquor extraction of tea tree and inferred that 30 s of microwaving diminished the required ethanolic extraction time and created oil most intently mirroring the oil inside the leaf. In a second report Pastry specialist and Firm (1995) found that for air-dried tea tree leaf (1 g) the ideal time for microwave started ethanolic extraction was 3 days [10].

As a rule, the science of basic oil can be clarified dependent on two significant gatherings, which are hydrocarbons and the

oxygenated mixes. The hydrocarbon bunch in fundamental oils comprises of the gathering monoterpenes, sesquiterpenes and diterpenes while the oxygenated mixes are assembled as esters, aldehydes, ketones, alcohols, phenols and oxides. *Melaleuca alternifolia* oil is contained mix blends of different monoterpenes, sesquiterpenes and their related alcohols, anyway as a generally, ruled by monoterpenes. As a rule, terpenes are named unstable and fragrant hydrocarbons are comprised of isoprene polymers, with a sub-atomic equation of C_5H_8 . As said beforehand, monoterpenes, arranged under the terpenes family, with an equation of $C_{10}H_{16}$, are known to be the primary fixings adding to the general organization of basic oils, explicitly in tea tree oil. In the prior stage, 12 segments were found, trailed by in 1965, 21 segments were perceived lastly, right around a sum of 100 parts were recognized, along with their relating structures in the wake of being tried with 800 examples of tea tree oil, both by means of GC and GC-MS characterization study. The identified monoterpenes in tea tree oil from the portrayal study are terpinen-4-ol, α -terpinene, β -terpinene, terpinolene, α -phellandrene, p-cymene, limonene, β -phellandrene, 1, 8-cineole (eucalyptol) just as the sesquiterpenes constituents comprising of aromadendrene, viridiflorene and λ -cadinene [11-13].

AUSTRALIAN TEA TREE PLANT (*MELALEUCA ALTERNIFOLIA* SP.) [14]

The Australian tea tree plant (*Melaleuca alternifolia* sp.); from the Myrtaceae. A fragrant and herbaceous plant variety, which is most popular for the creation of home grown fundamental oils because of its restorative and helpful constituents in the plant arrangements. Aside from its therapeutic worth, the rest of the parts from this plant are additionally valuable for different applications, for example, the branches from this plant are used for the brush fence creation, bark compositions, fixing and protection from their kaleidoscopic barks, fuel and development materials from their wood just as the nectar extricate from the nectar. At first, the presence of this plant was first found in the subtropical coastal district of New South Ribs in Australia by Commander James Cook during his exploratory journey in 1770, when he ran over a myrtaceous bush (most likely a *Leptospermum*, under the *Melaleuca* class) with leaves that were devoured by his mariners as a substitute for tea. The plantings of this herbaceous species were afterwards stretched out to the US, Zimbabwe, New Zealand, China, India and different pieces of the country. A full developed *Melaleuca alternifolia* sp. is set up to be a stature of inside 3 to 8 meters with a bush like development as appeared in Figure 01.



Figure 01. *Melaleuca alternifolia* shrub-like growths

Sankara Malathi et al., World Journal of Current Med and Pharm Research., Vol-II, Iss-IV, 271-279.

EXPERIMENTAL METHODOLOGY

PREPARATION OF PLANT SAMPLE [15]

The fresh tea tree plant sample was collected. Authenticated by the Government horticultural or agricultural university and were cleaned and oven dried at 40°C until a constant weight is achieved. The plant samples were later then segregated into smaller stem twiglets and were granulated into powder form by using a grinder. The powdered tea tree sample were then further refined through manual sieving of 1 mm mesh size and were stored into an air-tight container prior to the extraction process.

EXTRACTION PROCEDURES

Already, the regular work on being utilized for the extraction of tea tree oil from *M. alternifolia* plant is by utilizing the steam refining strategy. This traditional strategy is one of the antiquated methods of fundamental oil extraction, as the idea of extraction is professed to be immediate, least unpredictable and considered as outstanding amongst other technique to distil crude materials from plants (particularly leaves). By the by, with the headway of ebb and flow examination and innovation, different techniques are additionally being considered in this procedure, for example, dissolvable extraction strategy followed by progressive extraction, CO₂ supercritical extraction, microwave innovation, and furthermore enfleurage strategy. Be that as it may, for this venture purposes, just dissolvable extraction strategy is being underlined for the extraction study and will be clarified top to bottom in the accompanying segment.

I. STEAM DISTILLATION METHOD [16]

In this analysis, TTO were created by steam refining from the leaves and terminal parts of Australian *Melaleuca* trees. Dry materials were gotten following 24 hours of drying, and afterward decided the dampness of these materials (delegate inspecting). We refined both dry and wet materials to test effect of dampness in crude materials to fundamental oil sum separated. In the research facility scale, we considered steam refining strategies: utilizing small scale wave, and outside kettle. The primary gadget for this procedure is refining vessel of which measurements are 1110mm distance across, 1689mm tallness. The activity boundaries of this one are 120°C and 2 bars in 3 hours. In addition, steam refining process was additionally completed on standard hardware as indicated by standard of Vietnamese pharmacopeia gear. For steam refining process utilizing outside heater, steam limit is around 350 kg water for each hour. The steam stream rate into refining vessel is balanced naturally by controller associated with the temperature sensor and the weight sensor. Blend from condenser incorporates water and fundamental oil. As a result of the distinction of their particular gravity, they will become two fluid stages in the separator. The measure of basic oil that broke up in the distillate water is little (insignificant) and in this way recuperation of basic oil disintegrated in the water is pointless.

ESSENTIAL OIL REFINEMENT - VACUUM DISTILLATION PROCESS [16]

Unrefined TTO (35 – 45% of volume of terpinen-4-ol) from steam refining process were utilized as materials for this

investigation. In pilot scale, the boundaries of refining gear are 5cm inside measurement and 1.5m long segment which is filled by work cradle materials. To start with, feed the rough basic oils into the reboiler of the vacuum refining segment. At that point, the feeds are bubbled by roundabout warming resistor by means of warm oil warmer. A vacuum generator is associated with the head of refining pinnacle to make low-pressure in framework. Before the vacuum generator, we put the fluid separator, which is cooled by fluid nitrogen, so as to consolidate totally basic oil entrained by the vacuum line and to ensure this motor. The reflux proportion (R) was set by modifying On/Off time of solenoid valve to take TTO out. At the point when the valve closes (clock OFF), TTO fume was dense at the head of the pinnacle and made a reflux stream back to the pinnacle. On the other hand, when the valve opens (clock ON), TTO fume was brought to condenser. Since On/Off cycle is exceptionally short (in no time flat), it very well may be considered as persistent flow process. Two thermometers are put on the base and head of refining segment to decide every span precisely. The reason for TTO sanitization is to make item that has low grouping of 1, 8-cineole, high centralization of terpinen-4-ol. In this examination, we did in conditions: 6000ml of feed volume, 5mmHg and reflux proportions (part returned condensate to part condensate take off) was changed from 1 to 3. Vacuum distillation was shown in Figure 02.

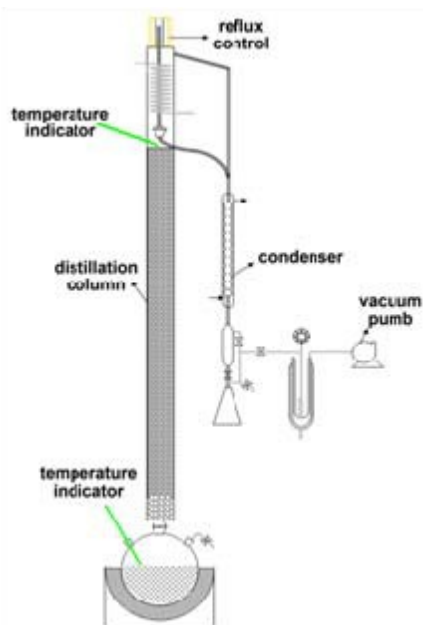


Figure 02: TTO refinement from Vacuum distillation

I. SOLVENT EXTRACTION METHOD [17,18,19]

50 g of refined tea tree powder test were estimated and moved into a thick cellulose thimble and were put into the Soxhlet extractor chamber. The extractor was then associated with a 500ml jar, containing 300mL of n-hexane, methanol, ethanol and oil ether dissolvable and was warmed at their breaking point temperature. The extraction procedure was proceeded for 6 extraction cycles (around 6 hours) and the extraction strategy was rehashed for oil ether, ethanol and methanol extraction.

After the extraction procedure, the gathered blend were then additionally sanitized utilizing turning evaporator under vacuum condition for the dissolvable expulsion process at 40oC, which at last gives a light yellow waxy surface, viewed as tea tree concrete. The solid buildup were then additionally

disintegrated in methanol dissolvable (6mL) to take out the common waxes and other substantial albuminous mixes and accordingly warmed at 40oC for 30 minutes, trailed by refrigeration at - 15oC for 24 hours, to accelerate the wax particles.

The blend is then sifted under vacuum filtration to recoup the blend of fundamental oils and methanol dissolvable from the waxes and different saps mixes. The filtrated blend were on the other hand cleansed under rotating dissipation to evacuate the overabundance methanol dissolvable, which at long last outcomes in the delivered fundamental oils along with certain hints of waxes and can be named as tea tree absolutes.

Solvent extraction from soxhlet apparatus was shown in Figure 3 and the final product was shown in Figure 04.

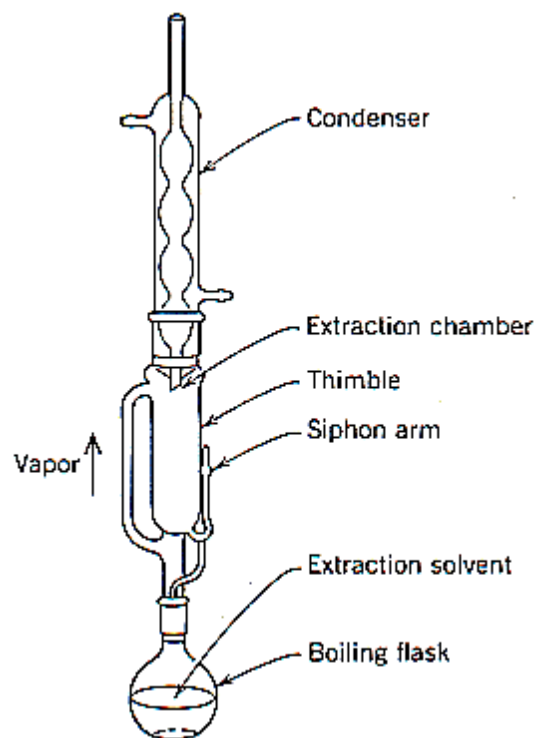


Figure 03: Soxhlet apparatus



Figure 04: Final TTO extract

SUCCESSIVE EXTRACTION

The methanolic separate (347 g) was oppressed for Soxhlet extraction in round bottomed carafe with oil ether (2.0 lts) for 12h. The concentrate was concentrated under diminished

tension at 50-60°C till total drying. The dried progressive oil ether extricate (yield 20 g, 5.76%) was put away in a shut vessel at 4°C in a cooler till further use.

The oil ether extricated leaf powder was dried and indeed exposed to Soxhlet extraction progressively with various solvents viz, chloroform, ethyl acetic acid derivation and methanol (2.0 lts each). The concentrates were thought and put away as portrayed previously. Yields, progressive chloro-form remove, 38 g, 10.95%, progressive ethyl acetic acid derivation extricate, 28 g, 8.06% and progressive methanol extraction 54 g, 15.54%. The yields were calculated in rate concerning the air-dried medication. The progressive concentrates were put away in shut vessels at 4°C in a cooler till further use.

DETERMINATION THE YIELD OF EXTRACTED TEA TREE ABSOLUTES

Once the recovery of tea tree absolutes is achieved, the mass of the end product is determined and hence, the yield of tea tree absolutes extracted based on different types of studied solvents can be determined using the expression below in Table 01.

$$\text{Yield of extract} = \frac{\text{Weight of extracted absolutes} \times 100\%}{\text{Weight of initial plant sample}}$$

EXTRACTION SOLVENTS	MASS OF EXTRACTED TEA TREE ABSOLUTES (G/G)	YIELD OF ABSOLUTES (%)
n-hexane	0.48	0.96
petroleum ether	0.53	1.06
Ethanol	0.39	0.78
Methanol	0.39	0.78

Table 01: Yield of absolutes from *Melaleuca alternifolia* sp. based on different types of solvents

It is claimed that petroleum ether extraction provides the highest yield of tea tree absolutes in comparison to the yield extracted from n-hexane, ethanol and methanol extraction.

II. MICROWAVE TECHNOLOGY [20]

A microwave-helped dry technique for separating basic oils was depicted by Craveiro et al. (1989). The oil created from *Lippia sidoides* was subjectively like the steam refined oil however essentially unique quantitatively. Solid (1995) and Southwell et al. (1995) have utilized microwave-helped dissolvable extraction for the fast GC examination of tea tree leaf tests down to 1 mg. They found that 10 s of microwaving diminished extraction time for tea tree leaf from 30 h to 1 h. They likewise analyzed the potential for a microwave pretreatment to liquor extraction of tea tree and presumed that 30 s of microwaving diminished the required ethanolic extraction time and created oil most intently mirroring the oil inside the leaf. In a second report Pastry specialist and Solid (1995) found that for air-dried tea tree leaf (1 g) the ideal time for microwave started ethanolic extraction was 3 days.

I. SUPERFICIAL FLUID EXTRACTION METHOD [21]

CHEMICALS

Carbon dioxide, SFE, methanol, n-hexane & absolute ethanol (analytical-reagent grade).

SAMPLES

Leaf samples (*M. alternifolia*) were randomly obtained from a small plantation at the Horticulture Precinct, Faculty of Science, Technology and Agriculture, University of Andhra Pradesh. For experiments involving dried leaf samples, the leaves were air dried at room temperature over a period of at least two weeks before any extraction work was carried out. For fresh leaves, samples were extracted within an hour of sampling. A commercial tea tree oil sample was provided courtesy of ATTORI (Australian Tea Tree Oil Research Institute, Lismore, Australia).

SAMPLE CRUSHING

Leaf material was transferred into a mortar and a sufficient quantity of liquid nitrogen added.

Grinding with a pestle was undertaken until a coarse appearance resulted.

SAMPLE REHYDRATION

A sufficient quantity of the leaf material was immersed in water overnight. On the following day, the sample was blotted dry prior to extraction.

SUPERCRITICAL FLUID EXTRACTOR AND EXTRACTION CONDITIONS

A Hewlett Packard (HP) 7680T SFE instrument, with accompanying software, was used throughout this study as an integrated system of extraction, subsequent trapping and analyte recovery. A known amount of the leaf material (approximately 100 mg) or a drop of the oil on filter paper was loaded into the sample chamber (thimble) between glass-fiber impregnated filter papers. Extraction procedures involved the manipulation of only two parameters for each sample type, (1) the chamber temperature and (2) the sc CO₂ density. For every chamber temperature/scCO₂ density combination employed, three successive extractions were performed on the same sample. A complete list of extraction parameters are outlined in Table 01. The extraction time (dynamic extraction) parameter in Table 01 was listed in terms of the number of thimble volume sweeps. The actual time required for this extraction mode varies with the scCO₂ density used; hence the volume sweep number is more informative for this work. The trap temperature was set at 5°C to minimize the loss of volatile components. SFE parameters were tabulated and shown in Table 02.

EXTRACTION CONDITIONS	VALUES
CO ₂ flow rate (mL/min)	1
Equilibrium time (min)—Static Extraction	10
Extraction time —Dynamic Extraction in number of times thimble volume sweeps	10 times
Nozzle extraction temperature (°C)	45
Trap extraction temperature (°C)	5
Trap packing	Octadecylsilane
Rince conditions	
Nozzle rinse temperature (°C)	45
Trap extraction temperature (°C)	5

Rinse solvent	Hexane
Rinse solvent flow rate (mL/min)	2
Rinse solvent volume (mL)	1.5
Void volume compensation (mL)	1.04

Table 02: SFE parameters that were maintained in all extractions.

PHYTOCHEMICAL SCREENING [22]

Phytochemical are primary and secondary metabolites that are presence in plants. Proteins and common sugars are part of primary components, whereas alkaloids and phenolic compounds are example of secondary components. Therefore, phytochemical test was done to detect the presence of these natural compounds. Phytochemical screening of TTO were listed in Table 03.

TEST FOR CARBOHYDRATES

To 2 ml of plant extract, 1 ml of Molisch's reagent, and a few drops of concentrated sulfuric acid were added. The presence of purple or reddish indicates the presence of carbohydrates.

TEST FOR TANNINS

To 1 ml of plant extract, 2 ml of 5% ferric chloride was added. Formation of dark blue or greenish black indicates the presence of tannins.

TEST FOR SAPONINS

To 2 ml of plant extract, 2 ml of distilled water was added and shaken in a graduated cylinder for 15 minutes lengthwise. Formation of 1 cm layer of foam indicates the presence of saponins.

TEST FOR FLAVONOIDS

To 2 ml of plant extract, 1 ml of 2N sodium hydroxide was added. The presence of yellow indicates the presence of flavonoids.

TEST FOR ALKALOIDS

To 2 ml of plant extract, 2 ml of concentrated hydrochloric acid was added. Then, a few drops of Mayer's reagent were added. The presence of green or white precipitate indicates the presence of alkaloids.

TEST FOR QUININES

To 1 ml of extract, 1 ml of concentrated sulfuric acid was added. Formation of red indicates the presence of quinones.

TEST FOR GLYCOSIDES

To 2 ml of plant extract, 3 ml of chloroform and 10% ammonia solution was added. Formation of pink indicates the presence of glycosides.

TEST FOR CARDIAC GLYCOSIDES

To 0.5 ml of extract, 2 ml of glacial acetic acid and a few drops of 5% ferric chloride were added. This was under-layered with 1 ml of concentrated sulfuric acid. Formation of brown ring at the interface indicates the presence of cardiac glycosides.

TEST FOR TERPENOIDS

To 0.5 ml of extract, 2 ml of chloroform was added and concentrated sulfuric acid was added carefully. Formation of

red-brown at the interface indicates the presence of terpenoids.

TEST FOR PHENOLS

To 1 ml of the extract, a few drops of phenol Ciocalteu reagent were added followed by few drops of 15% sodium carbonate solution. Formation of blue or green indicates the presence of phenols.

TEST FOR COUMARINS

To 1 ml of extract, 1 ml of 10% NaOH was added. Formation of yellow indicates the presence of coumarins.

STERIODS AND PHYTOSTEROIDS

To 1 ml of plant extract, equal volume of chloroform is added and subjected with few drops of concentrated sulfuric acid appearance of brown ring indicates the presence of steroids and appearance of bluish-brown ring indicates the presence of phytosteroids.

PHLOBATANNINS

To 1ml of plant extract, few drops of 2% HCL were added, and appearance of red precipitate indicates the presence of phlobatannins.

ANTHRAQUINONE

To 1 ml of plant extract, few drops of 10% ammonia solution were added, and appearance pink precipitate indicates the presence of anthraquinones.

SL. NO	PHYTOCHEMICAL TEST	TEE TREE OIL
1	Carbohydrates Test	+
2	Tannins test	+
3	Saponins test	Weakly +
4	Flavonoids test	-
5	Alkaloid test	-
6	Quinones test	+
7	Glycosides test	-
8	Cardiac glycosides test	+
9	Terpenoids test	+
11	Phenols test	+
12	Coumarins test	-
13	Steroids and phytosteroids	Steroids
14	Phlobatannins test	-
15	Anthraquinones test	-

CHEMICAL COMPOSITION OF TEA TREE OIL [23]

Tea Tree Oil from *Melaleuca alternifolia* contains different mono-and sesquiterpenes just as sweet-smelling mixes. The monoterpenes terpinen-4-ol, α -terpinene, α -terpinene, 1, 8 cineole, p-cymene, α -terpineol, α -pinene, terpinolene, limonene and sabinene represent 80 - 90% of the oil. The regular substance of the individual terpenes in Tea Tree Oil may change extensively relying upon the *Melaleuca alternifolia*

populace utilized, the atmosphere, the leaf maceration, the age of the leaves and the term of refining. Its significant constituents are introduced in Table 04. The substance structures for these significant constituents are appeared in

CONSTITUENTS	MINIMUM	MAXIMUM
Terpinolene	1.5	5
1,8-Cineole (eucalyptol)	Trace	15
α -Terpinene	5	13
γ -Terpinene	10	28
p-Cymene	0.5	8
Terpinen-4-ol	30	40
α -Terpineol	1.5	8
Limonene	0.5	1.5
Sabinene	Trace	3.5
Aromadendrene	Trace	3
δ -Cadinene	Trace	3
Globulol	Trace	1
Viridiflorol	Trace	1
α -Pinene	1	6
Ledene (syn. Viridiflorene)	Trace	3

**Table 04. Main constituents of Tea Tree Oil
(From ISO 4730-2004)**

ANTIOXIDANT ASSAYS [24-26]

NITRIC OXIDE RADICAL INHIBITION ASSAY

Sodium nitroprusside in a fluid arrangement at physiological pH immediately creates nitric oxide; it cooperates with oxygen to deliver nitrite particles, which can be assessed by the utilization of Griess-Ilosvay response (Garrat, 1964). In the current examination, Griess-Ilosvay reagent was changed utilizing naphthyl ethylenediamine dihydrochloride (0.1% w/v) rather than 1-naphthylamine (5%). The response blend (3 ml) containing sodium nitroprusside (10 mM, 2 ml), phosphate support saline (0.5 ml), and various centralizations of oils (200-600 μ g) or standard arrangement (0.5 ml) were brooded at 25°C for 150 minutes.

After hatching, 0.5 ml of the response blend containing nitrite was pipetted and blended in with 1 ml of sulfanilic corrosive reagent (0.33% in 20% cold acidic corrosive) and permitted to represent 5 minutes for finishing diazotization. At that point, 1 ml of naphthyl ethylenediamine dihydrochloride (1%) was included, blended, and permitted to represent 30 minutes. A pink-hued chromophore was shaped in diffused light. The absorbance of these arrangements was estimated at 540 nm against the comparing clear. Ascorbic corrosive was utilized as positive control. The scavenging activity was calculated using the formula.

$$\% \text{ of Inhibition} = (A \text{ of control} - A \text{ of test}) / A \text{ of control} * 100$$

1-DIPHENYL 2-PICRYLHYDRAZYL (DPPH) ASSAYS

The cell reinforcement action of the concentrates was estimated based on the rummaging action of the steady 1, DPPH free radical as per the strategy depicted by Brand-Williams et al. with slight alterations. 1 ml of 0.1 mM DPPH arrangement in methanol was blended in with 1 ml of fundamental oil arrangement of fluctuating fixations (200, 400, and 600 μ g). Relating clear example were readied, and L-Ascorbic corrosive (1-100 μ g/ml) was utilized as reference standard. Blender of 1 ml methanol and 1 ml DPPH arrangement was utilized as control. The lessening in absorbance was estimated at 517 nm following 30 minutes in dull utilizing a Bright obvious spectrophotometer. The inhibition % was calculated using the following formula.

$$\% \text{ of Inhibition} = (A \text{ of control} - A \text{ of Test}) / A \text{ of control} * 100$$

HYDROGEN PEROXIDE SCAVENGING ASSAY

The capacity of the concentrate to search hydrogen peroxide was resolved by the technique for Ruch et al. (1989). An answer of hydrogen peroxide (40 mM) was set up in phosphate cradle (pH 7.4). Hydrogen peroxide focus was resolved spectrophotometrically retention at 230 nm (8500 II, Bio-Crom GmbH, Zurich, Switzerland). Concentrates (200-600 μ g) in refined water were added to a hydrogen peroxide arrangement (0.6 ml, 40 mM). Absorbance of hydrogen peroxide at 230 nm was resolved following 10 minutes against a clear arrangement containing phosphate cushion without hydrogen peroxide. The percentage of scavenging of hydrogen peroxide of extract, and standard was calculated using the following equation:

$$\% \text{ of inhibition} = (A \text{ of control} - A \text{ of test}) / A \text{ of control} * 100$$

ANTIVIRAL ACTIVITY [27]

The antiviral movement of TTO was first demonstrated utilizing tobacco mosaic infection and tobacco plants. In field preliminaries with *Nicotiana glutinosa*, plants were showered with 100, 250, or 500 ppm TTO or control arrangements and were then tentatively tainted with tobacco mosaic infection. Following 10 days, there were essentially less sores per square centimeter of leaf in plants rewarded with TTO than in controls. Next, Schnitzler et al. analyzed the action of TTO and eucalyptus oil against herpes simplex infection (HSV). The impacts of TTO were researched by brooding infections with different centralizations of TTO and afterward utilizing these rewarded infections to contaminate cell monolayers. Following 4 days, the quantities of plaques shaped by TTO-rewarded infection and untreated control infection were resolved and thought about. The grouping of TTO repressing half of plaque development was 0.0009% for HSV type 1 (HSV-1) and 0.0008% for HSV-2, comparative with controls. These investigations additionally demonstrated that at the higher convergence of 0.003%, TTO diminished HSV-1 titers by 98.2% and HSV-2 titers by 93.0%. Moreover, by applying TTO at various stages in the virus replicative cycle, TTO was appeared to have the best impact on free infection (before disease of cells), in spite of the fact that when TTO was applied during the adsorption time frame, a slight decrease in plaque development was additionally observed. Another investigation assessed the exercises of 12 fundamental oils, including TTO, for movement against HSV-1 in Vero cells. Once more, TTO was found to apply a large portion of its antiviral movement on free infection, with 1% oil hindering plaque development totally and 0.1% TTO lessening plaque arrangement by roughly 10%. Pretreatment of the Vero cells

preceding infection expansion or posttreatment with 0.1% TTO after viral retention didn't altogether modify plaque development. Some movement against bacteriophages has additionally been accounted for, with presentation to half TTO at 4°C for 24 h lessening the quantity of SA and T7 plaques shaped on gardens of *S. aureus* and *E. coli*, separately. The aftereffects of these investigations show that TTO may act against encompassed and nonenveloped infections, despite the fact that the scope of infections tried to date is extremely restricted.

ANTIPROTOZOAL ACTIVITY [28]

Two distributions show that TTO has antiprotozoal action. TTO caused a half decrease in development (contrasted with controls) of the protozoa *Leishmania major* and *Trypanosoma brucei* at convergences of 403 mg/ml and 0.5 mg/ml, separately. Further examination indicated that terpinen-4-ol contributed essentially to this movement. In another examination, TTO at 300 mg/ml murdered all cells of *Trichomonas vaginalis*. There is additionally narrative in vivo proof that TTO might be compelling in rewarding *Trichomonas vaginalis* diseases.

ANTIMICROBIAL COMPONENTS OF TTO [29, 30]

Extensive consideration has been paid to which segments of TTO are answerable for the antimicrobial movement. Early signs from RW coefficients were that a significant part of the movement could be ascribed to terpinen-4-ol and α -terpineol. Information accessible today affirm that these two parts contribute generously to the oil's antibacterial and antifungal exercises, with MICs and MBCs or MFCs that are commonly equivalent to, or marginally lower than values acquired for TTO. Be that as it may, α -terpineol doesn't speak to a noteworthy extent of the oil. Extra parts with MICs like or lower than those of TTO incorporate β -pinene, γ -pinene, and linalool, be that as it may, like the case for α -terpineol, these segments are available in just generally low sums. Of the rest of the parts tried, it appears that most have probably some level of antimicrobial action, and this is thought to associate with the nearness of useful gatherings, for example, alcohols, and the dissolvability of the segment in natural layers. While some TTO parts might be viewed as less dynamic, none can be viewed as inert. Moreover, methodological issues have been shown to impact test results. The likelihood that parts in TTO may have synergistic or opposing associations has been investigated in vitro, however no critical connections were found. The likelihood that TTO may act synergistically with other basic oils, for example, lavender and other fundamental oil parts, for example, α -triketones from manuka oil has additionally been examined. Given the various parts of TTO, the extension for such impacts is colossal, and considerably more work is required to inspect this inquiry.

ANTI-ACNE VULGARIS (31)

This twofold visually impaired clinical preliminary was performed all out a year. An all out 1 of 60 patients (age run 15 to 25 years) with mellow to direct facial skin break out vulgaris. Moral advisory group leeway was taken before playing out this examination.

We played out this investigation with 60 patients in each gathering having 30. Patients were chosen arbitrarily and named them as gathering An and B. presently bunch A were treated with 5% tea tree oil gel and gathering B with fake

treatment gel. This fake treatment gel was comprised of carbomer gel and this was not having hostile to skin break out movement. Tea tree oil & fake treatment gels having a similar shading, surface, packaging size however, unique in names. Each patient was met with actually so they were no ready to contrast their medications and every other. The patient were told to apply the medication or fake treatment twice a day by day over the influenced regions for 20 mints and afterward wash it off to the typical faucet water. The treatment was preceded for 45 days. The two specialists and the patients were blinded to the kind of treatment. The patients were seen at each multi day time frame to assess the sores and any reactions. To decide the viability on skin break out seriousness we utilized both all out sore checks.

(TLC) and the acne severity index (ASI).

The ASI was calculated as:

ASI = Papules + (2×pustules) + (comedones /4)

The TLC count was calculated as:

TLC = Papules + pustules + comedones + nodules

The primary outcome measure was defined as the change in mean TLC and ASI scores at the end of treatment compared to baseline in both the study and control groups. Secondary outcome measures included a change in the mean numbers of comedones, papules and pustules.

A second investigator blinded to the type of treatment was responsible for counting the lesions before and after the treatment. In the first visit, the total number of lesions was considered to be 100% and any decrease in the number of lesions was calculated accordingly and regarded as the percentage of improvement. The mean of these percentages of improvement was calculated in each group of patients and used for statistical analysis. At the end of the study, the data were analyzed by the third investigator using SPSS (release 13) program (student's t test) and then the labels were revealed.

ANTI-INFLAMMATORY ACTIVITY [32, 33]

Various ongoing investigations presently bolster the recounted proof ascribing calming action to TTO. In vitro work in the course of the most recent decade has shown that TTO influences a scope of insusceptible reactions, both in vitro and in vivo. For instance, the water-dissolvable segments of TTO can hinder the lipopolysaccharide-actuated creation of the incendiary middle person's tumor putrefaction factor alpha (TNF- α), interleukin-1 (IL-1), and IL-10 by human fringe blood monocytes by around half and that of prostaglandin E2 by about 30% after 40h. Further assessment of the water-dissolvable portion of TTO distinguished terpinen-4-ol, α -terpineol, and 1, 8-cineole as the principle parts, yet of these, just terpinen-4-ol had the option to lessen the creation of TNF- α , IL-1, IL-8, IL-10, and prostaglandin E2 by lipopolysaccharide-initiated monocytes. The water-dissolvable part of TTO, terpinen-4-ol, and α -terpineol likewise stifled superoxide creation by agonist-invigorated monocytes yet not neutrophils. Interestingly, comparable work found that TTO diminishes the creation of responsive oxygen species by both invigorated neutrophils and monocytes and that it additionally animates the creation of receptive oxygen species by nonprimed neutrophils and monocytes. TTO neglected to smother the adherence response of neutrophil initiated by TNF- α incitement or the casein-prompted enlistment of neutrophils into the peritoneal cavities of mice. These investigations distinguish explicit systems by which TTO may act in vivo to decrease the typical incendiary reaction. In vivo,

topically applied TTO has been appeared to regulate the edema related with the efferent period of a contact extreme touchiness reaction in mice yet not the advancement of edema in the skin of nonsensitized mice or the edematous reaction to UVB presentation. This movement was ascribed basically to terpinen-4-ol and α -terpineol. At the point when the impact of TTO on excessive touchiness responses including pole cell degranulation was inspected in mice, TTO and terpinen-4-ol applied after histamine infusion decreased histamine-initiated skin edema, and TTO likewise fundamentally diminished expanding instigated by intradermal infusion of compound 48/80. Human examinations on histamine-prompted wheal and flare gave additional proof to help the in vitro and creature information, with the effective utilization of perfect TTO fundamentally diminishing mean wheal volume however not mean flare zone. Erythema and flare related with nickel-initiated contact extreme touchiness in people are additionally decreased by flawless TTO however not by a 5% TTO item, item base, or macadamia oil. Work has now indicated that terpinen-4-ol, however not 1, 8-cineole or α -terpineol, adjusts the vasodilation and plasma extravasation related with histamine-actuated aggravation in people.

CONCLUSION

This review clarifies the widespread applications of Tea tree (*Melaleuca alternifolia*) oil in cosmetics, healthcare and antiseptic products due to its antibacterial, antifungal, antiviral, anti-inflammatory, anti-acne, anti-protozoal and analgesic properties so on. Thus Tea tree (*Melaleuca alternifolia*) oil is highly significant as a naturally available medicinal plant extract. Future more studies was exploited in this TTO.

REFERENCES

- Carson CF, Hammer KA, Riley TV. 2006. *Melaleuca alternifolia* (tea tree) oil: a review of antimicrobial and other medicinal properties. *Clin. Microbiol. Rev.* 19:50-62.
- Altman, P. M. 1988. Australian tea tree oil. *Aust. J. Pharm.* 69:276-278.
- Sunita Lahkar¹, Malay Kumar Das², Sudarshana Bora³, An Overview on Tea Tree (*Melaleuca Alternifolia*) Oil, *International Journal of Pharmaceutical and Phytopharmacological Research (eIJPPR)*, Res. 2013; 3 (3): 250-253.
- Brophy, J. J.; Davies, N. W.; Southwell, I. A.; Stiff, I. A.; Williams, L. R. Gas Chromatographic Quality Control for Oil of *Melaleuca* Terpinen-4-ol Type (Australian Tea Tree). *J. Agric. Food Chem.* 1989, 37, 1330-1335.
- I. Southwell and R. Lowe, Tea Tree: The Genus *Melaleuca*, Wollongbar, Australia: *Harwood Academic Publishers*, 1999.
- Geofrey R.Davis, 1999 *Tea Tree Oil Distillation (Australia: Harwood Academic)* pp 155-168.
- Gagan Shah^{1*}, Dhandeep Singh², Uttam Singh Baghel³, Isolation and Identification of Compounds from the Leaf Extract of *Melaleuca alternifolia*, *Pharmacogn J.* 2017; 9(6)Suppl: s52-s55.
- Q Huynh¹, 2, T D Phan¹, V Q Q Thieu¹, 2, S T Tran² and S H Do², Extraction and refining of essential oil from Australian tea tree, *Melaleuca alterfornia*, and the antimicrobial activity in cosmetic products, *Asia-Pacific Inter disciplinary Research Conference 2011 (AP-IRC2011)*.
- K.A. Hammer et al 2006 A review of the toxicity of *Melaleuca alternifolia* (tea tree) oil, *Food and Chemical Toxicology* 44 616-62.
- Southwell, I. A.; Maddox, C. D. A.; Zalucki, M. P. Metabolism of 1, 8-cineole in Tea Tree (*Melaleuca alternifolia* and *M. linariifolia*) by Pyrgo Beetle (*Paropsisterna tigrina*). *J. Chem. Ecol.* 1995, 21, 439-453.
- H. Webb, R. Lanfear, J. Hamill, W. J. Foley and C. Kulheim, "The Yield of Essential Oils in *Melaleuca alternifolia* (Myrtaceae) is Regulated through Transcript Abundance of Genes in the MEP Pathway," *PLOS ONE*, vol. 8, no. 3, pp. 1-8, 2013.
- D. Telange, Analytical Studies of Some Essential Oils: Extraction, Analysis, Identification Techniques, *Deutschland: LAP LAMBERT Academic Publishing*, 2013.
- J. Rodney, J. Sahari and M. K. Mohd Shah, "Review: Tea Tree (*Melaleuca Alternifolia*) As A New Material for Biocomposite," *Journal of Applied Science and Agriculture*, vol. 3, no. 10, pp. 21-39, 2015.
- Boland, D., Brooker, M., Chippendale, G., Hall, N., Hyland, B., Johnston, R., Turner, J. (2006). *Forest trees of Australia*. Australia: *CSIRO Publishing*.
- D. R. H. Lemos, E. d. C. Melo, R. P. Rocha, L. C. d. A. Barbosa and A. L. Pinheiro, "Influence of Drying Air Temperature on the Chemical Composition of the Essential Oil of *Melaleuca*," in *Reveng, Vicoso-Brazil*, 2012.
- Geofrey R.Davis 1999 *Tea Tree Oil Distillation (Australia: Harwood Academic)* pp 155-168.
- J. M. Danlami, A. Arsad and M. A. A. Zaini, "Characterization and process optimization of castor oil (*Ricinus communis* L.) extracted by the Soxhlet method using polar and non-polar solvents," *Journal of the Taiwan Institute of Chemical Engineers*, vol. 47, pp. 99-104, 2015.
- M. A. Khan and S.-U.-R. , "Extraction and Analysis of Essential Oil of *Rosa* species," *International Journal of Agriculture & Biology*, vol. 7, no. 6, pp. 973-974, 2005.
- P. K. Rout, D. Sahoo and L. Misra, "Comparison of extraction methods of *Mimosa elengi* L. flowers," *Industrial Crops and Products*, vol. 32, pp. 678-680, 2010.
- Southwell, I. A.; Maddox, C. D. A.; Zalucki, M. P. Metabolism of 1,8-cineole in Tea Tree (*Melaleuca alternifolia* and *M. linariifolia*) by Pyrgo Beetle (*Paropsisterna tigrina*). *J. Chem. Ecol.* 1995, 21, 439-453.
- Victor Wong*, S. Grant Wyllie, Charles P. Cornwell and Deidre Tronson, Supercritical Fluid Extraction (SFE) of Monoterpenes from the Leaves of *Melaleuca alternifolia* (Tea Tree), *Molecules* 2001, 6, 92-103.
- NURUL AFIAH AMANI BINTI ZAABA^{1*}, GAYATHRI R2, VISHNU PRIYA V2, *IN VITRO* FREE RADICAL SCAVENGING ACTIVITY OF TEA TREE OIL AND CLOVE OIL, *Asian J Pharm Clin Res*, Vol 9, Suppl. 1, 2016, 179-183.
- Dr. C. Chambers, Prof. G. Degen, Dr. B. Jazwiec-Kanyon, Prof. V. Kapoulas, Prof. J.-P. Marty, Opinion on tea tree oil, *SCCP/1155/08*

24. Garrat DC. The Quantitative Analysis of Drugs. Vol. 3. Japan: Chapman and Hall Ltd.; 1964. p. 456-8.
25. Brand-williams W, Cuvelier ME, Berset C. Use of free radical method to evaluate antioxidant activity, *Lebensm Wiss Technol* 1995; 28(1):25-30.
26. Ruch RJ, Cheng SJ, Klaunig JF. Prevention of cytotoxicity and inhibition of intracellular communication by antioxidant catechins isolated from Chinese green tea. *Carcinogenesis* 1989; 10(6):1003-8.
27. Carson, C. F., and T. V. Riley. 1995. Antimicrobial activity of the major components of the essential oil of *Melaleuca alternifolia*. *J. Appl. Bacteriol.* 78:264-269.
28. Christoph, F., P. M. Kaulfers, and E. Stahl-Biskup. 2000. A comparative study of the in vitro antimicrobial activity of tea tree oils s.l. with special reference to the activity of α -triketones. *Planta Med.* 66:556-560
29. Griffin, S. G., S. G. Wyllie, J. L. Markham, and D. N. Leach. 1999. The role of structure and molecular properties of terpenoids in determining their antimicrobial activity. *Flav. Fragr. J.* 14:322-332.
30. Pen˜a, E. F. 1962. *Melaleuca alternifolia* oil—its use for trichomonal vaginitis and other vaginal infections. *Obstet. Gynecol.* 19:793-795.
31. Shahla Enshaieh, Abolfazl Jooya, Amir Hossein Siadat*, Fariba Iraj, The efficacy of 5% topical tea tree oil gel in mild to moderate acne vulgaris: A randomized, double-blind placebo-controlled study, [Downloaded free from <http://www.ijdv.com> on Friday, July 3, 2020, IP: 106.200.164.79].
32. Khalil, Z., A. L Pearce, N. Satkunanathan, E. Storer, J. J. Finlay-Jones, and P. Hart. 2004. Regulation of wheal and flare by tea tree oil: complementary human and rodent studies. *J. Investig. Dermatol.* 123:683-690.
33. Blackwell, R. 1991. An insight into aromatic oils: lavender and tea tree. *Br. J. Phytother.* 2:26-30
34. Bourne, K. Z., N. Bourne, S. F. Reising, and L. R. Stanberry. 1999. Plant products as topical microbicide candidates: assessment of in vitro and in vivo activity against herpes simplex virus type 2. *Antiviral Res.* 42:219-226.