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A REVIEW ON CHEMICAL CONSTITUENTS AND VARIOUS PHARMACOLOGICAL ACTIVITIES POSSESSED BY MELALEUCA ALTERNIFOLIA

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ABSTARCT
Melaleuca alternifolia, is one of that plant which is blessed with so many properties. Anti-microbial property is possessed by this plant due to presence of essential volatile oil in it. Tea tree oil is confirmed with broad antimicrobial properties and hence it is used to treat various disorders like dermatoses, vaginitis and respiratory tract diseases. Because of this, it is used as one of the active therapeutic ingredient in most of topical formulations. In some parts of Australia, crushed leaves are used to treat cuts and wounds. They apply leaves paste directly on wounds as this shows excellent antiseptic property and prevent the spread of infection. The tea tree leaf has confirmed with powerful anti-fungal properties and is used effectively for the treatment of multiple dermatophytes. Study reveals that leaf extract possess the antioxidant property because of the presence of various secondary metabolites such as terpenoids, phenolic compounds, flavonoids and steroids. There are number of medicinal properties that are there in this plant such as it is used to treat the problem of dandruff. Because of its germicidal properties it is used to treat the vaginal infection. This oil shows significant action against the acne as it can easily penetrate the skin and unblock the sebaceous glands. In last, this can be concluded that Melaleuca alternifolia is a single herbal shrub with many advantages.

KEYWORDS: Melaleuca alternifolia, Antiseptic, Antimicrobial, Antifungal.

INTRODUCTION

Herbal plants are the best source of treatment that is utilized by plenty of people, the significant reason behind this, is that these are less toxic and rarely show any side effect in the body. Many researches are going on to check their efficacy and impact on the human body. Melaleuca alternifolia belongs to the Family Myrtaceae, which has about 130 genera and approximately 3800-5800 species of predominantly tropical and subtropical distribution. This plant exists in the habitat of low-lying, swampy, subtropical and coastal ground. This plant is characterized as evergreen small tree or tall shrub. Studies reveals that plant is capable of showing Antimicrobial, Antifungal, Antiseptic, Germicidal and many more properties. It is used in treatment of acne and is even utilized to overcome the problem of dandruff. This is also used for treatment of eczema, burns, wound and for nail fungus. This plant is enriched with secondary metabolites hence is even found to be effective for the treatment of cancer. This plant is the hub of many therapeutic properties and for the same reason it is used as one of the active ingredients in many products' preparation. This is one of the natural fibres therefore can also be used as bio compost so as to preserve the environment and to prevent the unnecessary damage to the environment. The organoleptic description of this plant includes that it has simple leaves. The shape of a leaf is ovate. The size of a leaf is around 5-8 cm in length and 2-3 cm in width. The colour of upper surface of leaf is dark green in colour whereas the lower one is light green in colour. This is because of direct sunrays that falls on upper surface. The odour of plant is characteristic. On the other hand, the taste of plant is stringent and bitter. The petiole of leaf is narrowly winged. The margin is serrate and the apex is obtuse. The surface of a leaf is glabrous.

MORPHOLOGY OF *MELALEUCA ALTERNIFOLIA*

This plant is native to Australia. Tea tree oil is extracted from the leaves and terminal branches of this species. It was initially explored by aboriginal tribal population on the north coast of New South Wales.[1] This is an evergreen tall shrub or small tree with bushy crown and whitish papery bark. The leaves are small to medium size with 10-35mm long and 1mm wide. They are decussate or ternate with pinnate to parallel venation. Flowers are white or solitary either in spikes or in clusters. They are having 5 sepals, and petals which are 2-3mm long. In the proximal region hypanthium is fused to ovary. Stamens appear in bundle of 30-60, filaments are fused, anthers are dorsified and versatile with parallel cells that open in

longitudinal slits. Female part known as style consists of ovary which is 3-celled, there are few numbers of ovules in it and they are 3-4mm in length. Fruit is woody with capsule shaped and have a diameter of 2-3mm. there are numerous numbers of seeds in fruit, they are present with thin testa and are unwinged.[2], [3]

Figure 1: Melaleuca alternifolia.

GENERAL REQUIREMENTS OF THE PLANT

This plant is adaptable to wide range of atmospheric conditions. To have maximum yield, there are many factors that play their curious role such as temperature, rainfall, humidity, month of plantation, month of harvest and plant density.[4] Studies have revealed that propagation of Melaleuca alternifolia can also be done through seedlings, cuttings and tissue culture technique.[5]

- **Rainfall and Climatic conditions**

This plant responds well to the warm temperature and a continuous supply of moisture. It is native to wet sub-tropical area. The best temperature for the growth of plant is 18-34˚C. The annual rainfall required by the plant ranges from 1000-1600mm. This temperature directly effects the growth of the plant and can also leads to variation in the quantity of oil within the plant. Studies proof that increase in humidity is directly proportional to the concentration of oil in the plant. This plant has the capacity to grow even in drought conditions, irrespective of its natural behaviour. In those circumstances the tree will shed its leaves and will grow again to its full length after the rainfall.[6]

- **Soil Management**

The tea tree can grow well in soil pH range of 4.5-5.5.[7] Deep sandy loam or friable loam soil is most favourable soil for the growth of the plant as they can hold the water well. On the other hand, shallow light sandy soil, heavy clay soil and acid sulphate soil is not appropriate for the growth of the plant. Addition of the fertilizer should be in accordance to the soil texture and the need of the soil, however, minimum application of fertilizers is recommended for the best growth of the plant. The attention needs to be paid that excess use of fertilizers would hamper or damage the ground water quality.

- **Plant spacing and population**

The studies suggest that when plants are planted densely, the yield is found to be high as they are capable of fighting with the weeds in a better way because of their full growth. Studies from Sabah and Malaysia suggests that when plants are planted at a close distance within the rows, higher biomass yield is observed with less weeding problem.[8] On the other hand, another study suggests that when plants are planted at narrow distance the concentration of oil is found to be higher.[9]

- **Weed Control**

During the initial establishment phase, the weed can affect the growth of tea tree. New plant seedlings have very soft root system with diameter of 2.5cm and shoot height of 10-15cm and in such circumstances they cannot compete well with the weeds. In addition, too much shade by the weeds would also have negative impact on the growth of tree. Trees which are planted in soil with low water holding capacity or in region which receives less rainfall, have to struggle a lot for water along with the weeds. Both the tree seedlings and weeds grow at the surface of 30cm [10] and weeds have good water intake ability in comparison to tree seedlings. The young tea tree seedlings bear the water stress and such extreme conditions can cause the death of the plant.[11]

- **Harvest**

When the plant attains the height of 2m, the plant is ready to harvest. The plant attain this height with in the 9 months of plantation. Dry season is considered as the best time to harvest. Once the canopy is fully developed, the tree is ready for harvesting. The cuts are made at about 15-30cm above the ground level, so that the stumps left behind can regrow themselves. Oil is extracted from twigs and leaves.

Timing is very important from harvesting point of view since if it changes then there are chances that the oil gets vaporized. As the tree enters into the stage of harvesting, it starts losing its lower leaves and stems starts getting thicken. For this reason only, the time frame is limited only to 24 hours. The advantage of harvesting the plant during dry season is that it would also prevent the plant from fungal infection.[12]

According, to the study first harvest of the tree can reach 20t/ha of fresh biomass weight.[17] In the second and third harvest, the fresh biomass which can be produced by trees reach up to 25t/ha and 30t/ha respectively. It is for sure that, such fresh growth per hector can only be obtained through good growing conditions and management. From this it can be calculated that from single harvest around 20,000kg tea tree are ready for harvest.[39]

- **Postharvest Distillation**

The tea tree oil is collected from leaves and terminal branches. The oil is obtained by passing the steam through the oil bearing parts of the plant. Woody part of the plant does not contain any oil content.[13] The colour of oil is pale green with the smell of camphor. The best method that is employed for the extraction of oil is steam distillation method. The advantages of using steam is that it is readily available, cheap, and non-hazardous, used at low pressure, and can be recycled. Through steam distillation, it is possible to extract the oil at the lowest temperature so that there are the least chances of any change in the composition of the oil. The oil obtained from Tea tree is a mixture of monoterpenes, sesquiterpenes and their alcohols. The monoterpenes constitute about 80-90% of the oil.

CHEMICAL COMPOSITION OF THE OIL

Basically the tea tree oil is composed of terpene hydrocarbons, mainly monoterpenes and sesquiterpenes and their associated alcohols. Terpenes are the polymers of isoprene which has the formula of C_5H_8. Basically terpenes are volatile and aromatic hydrocarbons. There are numerous researches that were conducted to know about chemical composition of tea tree oil. Starting from late 60's, study claims the presence of 12 components in the oil.[14] Then in late 80's, scientist confirms the presence of 48 components in the oil.[15] In 1989, the team of researchers confirmed that there are all together 100 components that are present in the oil. And all around 800 different samples were tested so as to ensure with their range.[16] The samples were tested using gas chromatography and GCMS technique. Finally, the international standard has regulated the standard of tea tree oil, in which its maximum and minimum range has been set. Moreover, they had given a name "oil of Melaleuca" trepinin-4-ol type.

CHEMICAL COMPONENTS INCLUDES[17], [18], [19]

Table 1: Chemical Composition Of *Melaleuca Alternifolia.*

S.no	Name of component	Type of compound	Chemical Formula
1.	Terpinen-4-ol	Monocyclic terpene alcohol	$C_{10}H_{18}O$
2.	γ-Terpinene	Monocyclic terpene	$C_{10}H_{16}$
3.	α-Terpinene	Monocyclic terpene	$C_{10}H_{16}$
4.	1,8-cineole	Monocyclic terpene alcohol	$C_{10}H_{18}O$
5.	α-Terpinolene	Monocyclic terpene	$C_{10}H_{16}$
6.	ρ-cymene	Monocyclic terpene	$C_{10}H_{14}$
7.	α-Pinene	Dicyclic terpene	$C_{10}H_{16}$
8.	α-Terpineol	Monocyclic terpene alcohol	$C_{10}H_{18}O$
9.	Aromadendrene	Sesquiterpene	$C_{15}H_{24}$
10.	δ-Cadinene	Sesquiterpene	$C_{15}H_{24}$
11.	(+)-Limonene	Monocyclic terpene	$C_{10}H_{16}$
12.	Sabinene	Dicyclic monoterpene	$C_{10}H_{16}$
13.	Globulol	Sesquiterpene alcohol	$C_{15}H_{26}O$

NOMENCLATURE CONSIDERATION

Tea tree name causes the much confusion, the most frequent one is with renowned tea plant (Camellia sinensis, Theaceae). This confusion also, persists for the other members of Myrtaceae, family. This problem is very common for the species belonging to the genus Melaleuca, genus Leptospermum, genus Kunzea and genus Baeckea, these are well grown species in Australia and New Zealand.[20] The main problem is due to the vernacular name "tea tree" which is collectively used for the plants which are also known as "Paperbark trees". And from very long time both the terms were extensively used for the plants belonging to the Melaleuc or Lectospermum genera. In New Zealand, the plants named Kunzea ericoides and Leptospermum scoparium which are commonly known as kanuka and manuka respectively, the essential oil is derived from them and locally they are known New Zealand tea tree oil. This is also confused up with the Melaleuca alternifolia, although their chemical composition is entirely different. Even in Australia, Leptospermum species is cultivated domestically and this is also taken up as a tea tree oil in spite of many physical and chemical changes.[21]

PHARMACOLOGICAL ACTIVITIES

Antiprotozoal Activity: Several studies has been conducted to prove that the tea tree oil has the ability to show antiprotozoal activity. When this oil has been tested against the Leishmania major and Trypanosoma brucei, it has been noted that there is 50% reduction in the growth of protozoa.[22] The same study was also performed against the cells of Trichomonas vaginalis cells, and it was noted that there was complete destruction of the cells. Through the in vivo studies it has been concluded that tea tree oil has effectively treated the Trichomonas vaginalis infection.[23], [24] Investigation has proved that the anti-protozoal activity is due to the presence of terpinin-4-ol, which is found to be effective to treat the infection caused by these protozoas.

Antimicrobial Activity: TTO was discovered to possess the antimicrobial properties by Abrogines of Northern New South Wales. For the treatment of cough and cold, the leaves were crushed and inhaled. Even for the treatment of wound, after the application of poultice the crushed leaves were sprinkled on it.[25] Infusion can also be prepared using the tea tree oil leaves so as to treat skin ailments and sore throats.[26] During research the TTO was compared with disinfectant such as carbolic acid or phenol through the test known as Rideal-Walker coefficient.[27] After the test, it was found that the activity of TTO was 11 times better in comparison to phenol. By that time, TTO was taken as one of the therapeutic agents.[28], [29], [30] The main agent behind the antimicrobial activity of TTO is still to discover but it was assumed through RW coefficients that it was due to that presence of terpinen-4-ol and α- terpineol as this was supported by recent studies conducted by researchers.[31], [32], [33] Through the in-vitro studies it was found that antimicrobial activity is might be due to the interaction with other essential oils such as lavender oil and due to this synergistic effect is observed.[34], [35]

Antiviral Activity: TTO, antifungal activity was checked keeping in view of Agricultural application. Study was conducted on *Nicotiniana glutinosa* plant using tobacco mosaic virus. The plants were deliberately infected with this virus and were observed for the period of ten days after the spray of TTO as well as the control. After that, it was found that fewer lesions were observed on the surface of leaf that were sprayed with TTO oil in comparison to control.[36] In 2001, the antiviral activity of TTO was checked along with eucalyptus oil against herpus simplex virus.[37] Before the activity was checked the virus was incubated with varying concentration of TTO, then that virus was utilized to infect the cell monolayers. After an interval of four to five days the virus treated with TTO and control, both were examined. And it was noted that plaque formation in virus treated with TTO was declined by 50%, in comparison to the control. Also when, TTO was applied at different stages of virus replicative cycle, TTO has shown the greatest effect on the free virus. During the adsorption phase also, it has shown the decrease in plaque formation. In another study, TTO was studied along with other 12 essential oils against the HSV-1 in vero cells.[38] And the result was almost same as to previous study in which TTO was found to be effective against free viruses and reduction in plaque formation. Through these studies it was observed that TTO has the potential to act against the enveloped and nonenveloped viruses, but still many researches need to be conducted to ensure with the main reason behind this activity in TTO.

Anti-inflammatory Activity: When TTO was applied topically, it reduces histamine-induced oedema of the type that arises due to the allergic hypersensitivities [40]. Through, in-vitro studies it has noted that TTO reduces normal inflammatory response. The basic reason behind this activity is terpinen-4-ol, which leads to inhibition of many inflammatory mediators.[41] It can also, suppresses the superoxide production to show its anti-inflammatory activity.[42] In the in-vivo-studies, it has been found that oedema that is due to the contact hypersensitivity response, even that can be modulated by applying TTO. The reason attributed for this is the presence of terpinen-4-ol and α-terpineol.[43]

Antioxidant Activity: Antioxidant activity of TTO was evaluated using the methanolic extract of the leaves of Melaleuca alternifolia. The extract was prepared by subjecting the leaf powder to soxhelt extraction using methanol as solvent. Then the extract was concentrated at the temperature of 50-60˚c until the extract is completely dried. Upon this extract various tests were performed such as DPPH radical scavenging activity, ABTS Radical Cation Scavenging Method, Scavenging of Superoxide radical by Alkaline DMSO Method and Nitric oxide radical inhibition. In each of these tests, the TTO was compared against the Ascorbic acid and then the absorbance is noted using the spectrophotometer. For DPPH radical scavenging activity, IC_{50} value was found to be more than 50% than the ascorbic acid, therefore the methanolic extract shows the significant DPPH radical scavenging activity.[44] For ABTS Assay, IC_{50} value of methanolic extract was found to be higher as compared to Ascorbic acid.[45] For Scavenging of Superoxide radical by Alkaline DMSO Method, IC_{50} value of methanolic extract of TTO was found to be around 89%, which is much higher in comparison to Ascorbic acid.[46] For Nitric oxide assay, in this assay also methanolic extract of Tea tree oil has shown excellent nitric oxide antioxidant activity in contrast to Ascorbic acid.[47] From the above results, it was clear that TTO has excellent antioxidant activities. This is all because of presence of some phytoconstituents in its oil. These were terpenoids, phenolic compounds, flavonoids and steroids.[48], [49] In each of DPPH, ABTS, NO, and SO assay the IC_{50} value for methanolic extract was found to be much better in comparison to Ascorbic acid. Therefore, this confirms with the presence of these constituents in TTO to show antioxidant properties.

Biocompost properties: Normally, after distillation either the tea tree leaves will be burnt off or they will be composted. There are many companies which dry the residue of leaves after distillation and return that to the ground to be used as mulch.[50] In addition to this, there are many firms in Australia which are utilizing these dried leaf residue as a boiler fuel and it was noted that only 30% of it is required to bring it on the another distillation process, which gives us an idea that it can be used as a fuel positive process. On the contrast, burning of such residues will enhance the environmental issues and need to be monitored and controlled at the earliest. In another study, it was concluded that burning and decomposing the leaves in-situ will not contribute for the better planation yield.[51], [52] And one should look out for another way out to discard this agricultural waste without effecting the environment.[53] Tea tree oil may have some left-over oil in them after distillation and because of this leaves may take a long time to get decomposed. Based upon these facts it gives us consideration that the residue of tea tree leaves can be utilised as Biocompost as these are enriched with the natural fibres. Keeping in mind the future aspect, scientists are trying to discover the zero waste and green technology aspect from every part of the plant.

Antibacterial Activity: From the early 1990s there are many reports that explain the activity of TTO as an anti-bacterial agent. Researchers used the agar method, to assess the anti-bacterial activity of TTO. Through these results they were confirmed that TTO has the potential to exhibit the anti-bacterial property. The significant mechanism of action is still not clear but they all relate to leakage of ions, lysis of cells and inhibition of respiration. It was observed that when TTO was tested against the S.aureus, it was noted that there was precipitation of potassium ions and hence causes the leakage of ions.[54], [55] And this, only inhibits the respiration in cell.[54] In another study, it was also observed that TTO causes the morphological changes in the cell.[56] These morphological changes cause the lysis of the cells and it was not sure whether that leads to complete lysis of cell or not when they were observed through spectrophotometer [56] or under the electron microscopy.[57] When bacterial cell was treated with TTO it was found that there was modest uptake of propidium iodide[58] and there was no cytoplasmic membrane damage through lactate dehydrogenase.[57] All in all, it was concluded that TTO has deteriorating effect on potassium homeostasis[59], morphology[60] and glucose dependent respiration and TTO[59], also have the ability to exclude propidium iodide. So, it can be concluded that TTO has both structural and functional changes in the cells of bacteria.

Antifungal Activity: There are many studies which confirms that TTO is susceptible to various Yeasts, dermatophytes and other filamentous fungi. However, there are different test pattern which were followed among them many, assay regarding anti-fungal activity were performed on fungal conidia, which is relatively found to be impervious to most of the chemical agents. These results suggest that germinated conidia are more susceptible to TTO in comparison to nongeminated conidia and this is due to the intact conidial wall that provides the considerable protection.[61] Through the other studies, it has also been demonstrated that TTO has the ability to inhibit the fungal growth[62], [63] and also affect the rate of sporulation [64]. The most prominent reason that was enlighten after the studies is that TTO might alters the permeability of the cells. It has also been noted that oil significantly alters the membrane properties and leads to inhibition of respiration process. Study also confirmed that TTO leads to the inhibition of glucose-induced medium acidification through expulsion of protons by plasma membrane ATPase, which is filled with energy derived from Mitochondria. This inhibition largely suggests that TTO, effects the mitochondrial membrane. This is considered as proposed mechanism of action for TTO to work as antifungal agent. This mechanism is further supported by the work in which terpene eugenol inhibits the working of mitochondria and leads to the depletion of energy within the fungal membrane.[65]

Onychomycosis Treatment: onychomycosis, also known as tinea unguium is a fungal infection of the nail. Generally, in this discoloration of nail occurs either to white or to yellow. TTO is effective against multiple dermatophytes affecting the skin.[66] There have been many studies that were conducted to check the therapeutic effect of the TTO for the treatment of onychomycosis.[67] Treatment of this is very tedious on its own as it involves the infection within the nails and it's very difficult to reach at the exact point of infection. For the TTO to be checked weather it is effective for treatment of fungal infection, minimum six months study need to be proceeded. The test and various changes in the nails need to be recorded at regular interval of one, three and six months. Data obtained from such analysis would enable us to understand the antifungal property of TTO.[68] By the end of study, it was observed that TTO was found to effective for treatment of Onychomycosis by the regular application of this for the interval of six months. And the most relaxing thing is that no serious side effect as noted because of the constant use of TTO. It was found to be safe and effective.

Side effects: Through the various studies it was assumed that TTO is therapeutically safe and effective to be used both for internal and external purposes. Although, it too have some limitations which includes allergic reactions, redness, inflammation and rashes. This is might be due to the presence of any one of its major constituent. On research, it was found that it was due to 1-8 cineole. Another reason noted for this is the continuous degradation and oxidation of TTO which leads to cause of these side effects. Therefore, one should think for potent delivery vehicle or mode through which the degradation of oil can be prevented. Along with this its

bioavailability can be enhanced with the increase in retention time on skin.[69]

CONCLUSION: It is clear that tea tree oil obtained from Melaleuca alternifolia is very potent in showing the positive impact against the various disorders. In present scenario, where the use of antibiotics is getting obsolete its necessary to find the way for the treatment of many infectious diseases. People are getting attracted towards the use of phytochemicals and herbal drugs that are directly or indirectly derived from plants. There are many significant reason behind this thought as these herbs are more potent in action, their efficacy is high, therapeutically active, safe and free from side effects. TTO has shown the presence of some phytoconstituents in it hence used as a good antioxidant agent. Because of presence of isoprene unit as a basic structural unit it is capable of showing antimicrobial, antifungal, antiprotozoal, antiviral, antibacterial activities. It consists of natural fibres in it, therefore it can even be employed as a biocompost agent. It can also be used as one of good candidate for the treatment of cancer. But, still there is more ways to go ahead in the research study of this plant so as to explore the best out of it.

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