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Research Article

ANTIOXIDANT ACTIVITY, TOTAL PHENOLIC CONTENT OF ESSENTIAL OILS, AND EXTRACT DETERMINED FROM NATURAL LEAVES, *IN-VITRO* LEAVES, AND CALLUS SOURCES OF MELALEUCA ALTERNIFOLIA – A COMPARATIVE STUDY

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ABSTRACT

Objectives: Essential oil and their compounds are getting increasing interest due to their multipurpose functional as alternatives to artificial preservatives. The aim of this study was focused to comparative analyses on hydrodistillation, total phenolic content (TPC), and antioxidant activity of essential oil derived from *Melaleuca alternifolia* (*M. alternifolia*) leaves procured from natural leaves, *in-vitro* leaves, and callus sources.

Methods: The essential oil was extracted using hydrodistillation. The Folin-Ciocalteu method was used to determine the TPC equivalent and antioxidant activity of essential oils.

Results: TPC of essential oil from *in-vitro* sources of leaves contained 14.79 mg gallic acid equivalent/g dry plant material and its IC_{50} value was found to be 70% in 1-diphenyl 2-picrylhyorazyl assay. Thus, this source of essential oil showed good free radical scavenging activity.

Conclusion: The estimated biological potential was obtained in the essential oil from *in-vitro* sources of leaves *M. alternifolia*.

Keywords: *Melaleuca alternifolia*, Essential oil, Hydrodistillation, Total phenolic content, 1-Diphenyl 2-picrylhyorazyl.

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INTRODUCTION

Many complementary and alternative medicines have enjoyed increased popularity in recent decades. One such product is tea tree oil (TTO), the volatile essential oil derived mainly from the Australian native plant *Melaleuca alternifolia*. The TTO is extracted through a steam distillation process. Nowadays, TTO is very famous, and it is used in cosmetic and health care. In general, it is used for treating ringworm and athlete's foot, cuts and scrapes, acne, dandruff, and skin infections such as herpes, wounds, warts, burns, insect bites, nail mycosis, colds, sore throat, gingival infections, hemorrhoids, and vaginal infections [1-5]. Besides, it also has other therapeutic properties such as anti-inflammatory, antioxidants, anticancer [6,7], antibacterial, antifungal, antiviral, and also analgesic properties [8-10]. Efforts to validate their use have seen their putative therapeutic properties come under increasing scrutiny *in-vitro* and, in some cases, *in-vivo*. Obtaining extracts from the *in-vitro* tissues is easier than that from complex tissues of a plant. Furthermore, the quality of the phytochemicals can be improved through *in-vitro* propagation of plants to produce potential medicinal compounds [11] and plant tissue culture techniques serve this opportunity in tailoring the chemical profile of the plant through manipulating the chemical or physical microenvironment. Known for its antimicrobial properties, TTO is incorporated as the active ingredient in many topical formulations widely available over the counter in Australia, Europe, and North America and is marketed as a remedy for various ailments [7] used to treat cutaneous infections. TTO from *M. alternifolia* is composed of various monoterpenes, sesquiterpenes, and aromatic compounds. From about 100 terpenes found in TTO, more than 60 individual substances have been identified. The monoterpenes terpinen-4-ol, γ-terpinene, α-terpinene, 1,8-cineole, p-cymene, α-terpineol, α-pinene, terpinolene, limonene, and sabinene account for 80–90% of the oil [12].

METHODS

Plant collection

The leaves of *M. alternifolia* were collected from the commercial plantations present at Idukki, Kerala, India. The species was authenticated in the Department of Botany, Holy Cross College (Autonomous), Tiruchirappalli, India and a voucher specimen are preserved in the herbarium of the department.

Preparation of plant material and extraction of essential oil

The fresh leaves of *M. alternifolia* (from natural, *in-vitro,* and *in-vivo* plant sources) were harvested (Fig. 1) and washed with water to obliterate dirt, and then the leaves were subjected to hydrodistillation for 6 h. The extracted essential oils were dried over anhydrous sodium sulfate to remove any trace of water and stored in sealed glass vials at 4°C until further analysis. The yield was calculated according to the volume of obtained essential oil and was expressed on the basis of fresh weight (v/w).

Determination of total phenolic content (TPC)

The determination of TPC was done by the method reported by Ibrahim *et al.* [13] with slight modifications. A mixture of sample solution (1 ml), Folin-Ciocalteu reagent (0.5 ml), and ultrapure water (5 ml) was incubated at room temperature (RT) for 5 min. Then, 1 ml Na_2CO_3 (5% w/v) solution was added and incubated for 60 min at RT in the dark. The absorbance of the above mixture was measured at 760 nm using ultraviolet (UV)-spectrophotometry. Ethanol (70%) and gallic acid were used as negative and positive controls, respectively. The TPC of the sample was compared to a gallic acid standard curve ($y = 0.011x + 0.014$; $R^2 = 0.996$) and expressed relative to the equivalent standard concentrations (mg GAE/g dw, expressed as TPC per g powder). All determinations were performed in triplicates.

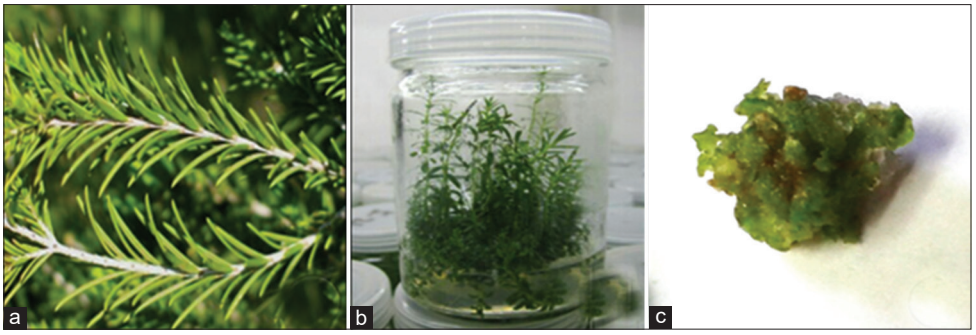

Fig. 1: *Melaleuca alternifolia* plant sources (a). Natural leaves (b). *In-vitro* leaves (c). Callus

Antioxidant assays

The antioxidant activity of the extracts was measured based on the scavenging activity of the stable 2,2-Diphenyl-1-picrylhydrazyl (DPPH) free radical assay [14] according to the method described by Williams *et al.* with slight modifications. One milliliter of 0.1 mM DPPH solution in methanol was mixed with 1 ml of essential oil solution of varying concentrations (200, 400, and 600 µg). The corresponding blank sample was prepared, and L-Ascorbic acid (1–100 µg/ml) was used as a reference standard. Mixture of 1 ml methanol and 1 ml DPPH solution was used as control. The decrease in absorbance was measured at 517 nm after 30 min in the dark using a UV-spectrophotometer. The percentage of inhibition was calculated as

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

RESULTS

Essential oil yield from natural leaves, *in-vitro* leaves, and callus sources of *M. alternifolia*

The yield of hydrodistillation essential oils from *M. alternifolia in-vitro* leaves was 50% (v/w) (Table 1) and it was characterized by a pale yellowish color and pleasant aromatic fragrance.

Estimation of TPC

As shown in Table 2, phenolic content TPC was estimated by the Folin-Ciocalteu colorimetric method using gallic acid as standard the mean value of TPC. The phenolic compounds are often correlated to the antioxidant activity due to their capability to act as electron donors in free radical reaction. The TPC quantifiable from *M. alternifolia in-vitro* leaves was (14.79 mg GAE/g dw).

DPPH assay

The antioxidant activity of TTO was analyzed using DPPH assay and ascorbic acid was taken as standard (Table 3). While essential oils from three different sources showed approximately equal inhibitory activity, the essential oil derived from *in-vitro* leaves of *M. alternifolia* showed 70% inhibition for the concentration of 600 µl. The concentration of essential oils with a concomitant increase in radical scavenging was observed and the values were recorded as a percentage of inhibition.

Table 1: Total yield of essential oils

S. No.	Sources of essential oils	Essential oil yield (ml/g plant)
1.	Natural leaves	0.49±0.11
2.	Callus	0.47±0.17
3.	*In-vitro* leaves	0.50±0.13

Each value represents mean±SD done in triplicates, p value<0.05 is considered significant

Table 2: TPC estimation

S. No.	Sources	TPC (mg GAE/g dw)
1.	Natural leaves	14.63±1.87
2.	Callus	14.71±1.96
3.	*In-vitro* leaves	14.79±1.62

TPC: Total phenolic content, Each value represents mean±SD done in triplicates, p value<0.05 is considered significant

Table 3: Antioxidant activity of essential oils

S. No.	Sources of essential oils	Concentration (µl)	Percentage of inhibition
1.	Natural leaves	600	58.52±1.31
2.	Callus	600	62.21±1.40
3.	*In-vitro* leaves	600	70.41±1.02

Each value represents mean±SD done in triplicates, p value<0.05 is considered significant

DISCUSSION

The yield of essential oils from leaves of *M. alternifolia* extracted by hydrodistillation method from the natural leaves, *in-vitro* leaves, and callus sources in the present study was in close agreement with other studies [15], where it has been reported with a similar yield of the essential oil from *M. alternifolia in-vitro* leaves was 0.50%. Indeed, the extraction of phenolic compounds from their natural matrix is complex by their diversity and their susceptibility to oxidation and hydrolysis. Similarly, several factors influence the amount of phenolic components such as variety, environmental conditions, and the mode of keep substrates extraction, the degree of ripening and genetic agents as well as many parameters related to the extraction methods such as temperature, time, solvent to solid ratio, and solvent type [16]. According to the present findings, the TPC of this oil was 14.79 mg GAE/g dw. TPC correlates with the antioxidant activity of the extract and our results were similar to those previously reported studies [17,18]. This essential oil is as good as a natural antioxidant exhibiting similar antioxidant activity resembling clove oil [19], due to their antioxidant activity; they are capable to scavenge the free radicals [20], which are dangerous for the body and could also possibly possess other therapeutic benefits such as anti-inflammatory, antibacterial, and anticancer activity [21-23].

CONCLUSION

Hence, they can be used in drug formulations, and more research can be carried out in the future to explore the medicinal properties of these oils.

AUTHORS' CONTRIBUTION

Ms. Jeyakani did all the experimental work and writing the article. Dr. Rajalakshmi did research guidance and critical revision of manuscript.

CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

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