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Chemical composition, antimicrobial and antioxidant properties of essential oils of *Trichopus zeylanicus* ssp. *travancoricus*

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Trichopus zeylanicus ssp. *travancoricus* is locally known as *Aroghyapacha* which means the leaves that give health and vitality. The present study aimed to analyze the chemical composition of essential oils from the leaves and swollen part of the petiole of *T. zeylanicus* ssp. *travancoricus* and to evaluate its antimicrobial and antioxidant potential. Essential oils were isolated from the leaves and swollen part of the petiole and analyzed by GC-MS. The major components of the leaf essential oil were α-humulene (48.99%) and β-caryophyllene (30.08%), whereas α-humulene (36.69%) and n-Hexadecanoic acid (17.41%) were the main components of the swollen part of petiole. The antioxidant activities were evaluated by DPPH and phosphomolybdenum assays. Both of the essential oils exhibited poor to moderate antioxidant activity (DPPH assay, IC50 >1000 µg/mL). The phosphomolybdenum assay of the leaves (1.70±0.01 mg equivalent AAE/g) and swollen part petiole (1.85±0.03 mg equivalent AAE/g) essential oils showed moderate total antioxidant capacity. Both the oils exhibited potent antimicrobial activity against Gram-negative bacteria than Gram-positive bacteria. The present study is a experimental proof of the previous attempts on ethnobotanical investigations carried out among Kani tribes of Agasthymalai Biosphere Reserve.

Keywords: Antimicrobial, *Aroghyapacha*, DPPH assay, GC-MS analysis, Kani Tribes, α-humulene.

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Introduction

Trichopus is an interesting genus, belongs to the family of Dioscoreaceae. The genus has two rhizomatous herbs, *Trichopus zeylanicus* Gaertn. and *T. sempervirens*. Of these two species, *T. zeylanicus* is recorded as native to Peninsular India, Sri Lanka, Malay Peninsula, Singapore, and Thailand whereas, *T. sempervirens* has a restricted distribution in Madagascar. *T. zeylanicus* have three subspecies namely *T. zeylanicus* ssp. *angustifolius*, *T. zeylanicus* ssp. *travancoricus* and *T. zeylanicus* ssp. *zeylanicus*. This species is mostly confined near the wet banks of streams and rivulets of the dense forests. In India, *T. zeylanicus* ssp. *travancoricus* has been located only in the Agasthyamalai Biosphere Reserve of southern Western Ghats[1-3].

T. zeylanicus ssp. *travancoricus* Burkill ex K. Narayanan is known as *Aroghyapacha* (Malayalam) or *Arogyapachai* (Tamil) by the Kani tribe which means that the green that gives strength[4]. It has been used by Kani tribes for rejuvenating purposes and also used as medicine for various diseases. The Kani tribe claim that unripened fruits soaked in honey for ten days and consumed orally gives relief from asthma[5]. Also, the plant exhibited potential activity in combination with other plants. Likewise, the leaves of this plant are taken along with the stem bark of *Mangifera indica* to treat venereal diseases[5]. Powder of the whole plant of *Diospyros ebenum*, leaves and fruits of *T. zeylanicus*, rhizome of *Curculigo orchioides*, and fruits of *Phyllanthus emblica* and *Terminalia bellirica* were taken orally along with honey to strengthen the body. These combinations of herbals were reported to contain phytochemical constituents such as ceryl alcohol, lupeol, betulin, sitosterol, diospyric acid, triterpene, and carboxylic acid[6]. The Kani tribe has contributed to bringing the multifarious uses of this wild plant to the present medicine world. This wonder herb also has several other pharmacological activities and medicinal properties such as choleretic, hepatoprotective, mast cell stabilization, aphrodisiac[7,8,9], adaptogenic[10], and cardioprotective[11]. The whole plant has the potential for anxiolytic and hepatoprotective activity[4], immunomodulatory activity[12] and anti-ulcer activity[13].

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The bioactive compounds of *T. zeylanicus* ssp. *travancoricus* were reported as potentially active in various medicinal treatments and can be used for the treatment of various diseases[14].

The literature review revealed that the research on essential oil isolation and analysis of its biological properties are lacking. From this notion, the present investigation could be assumed as the first report on the antioxidant and antimicrobial activities of essential oil of this species. The aim of this study was to determine the chemical compositions of essential oil of leaves and swollen part of petiole and to compare the antioxidant and antimicrobial activities.

Materials and Methods

Collection of plant material

Fresh leaves and petiole were collected during their leaf flushing season (6th November 2016). The collected specimen were identified by Dr Raju Ramasubbu, Assistant Professor and Gandhigram University Dindigul (GUD) -Herbarium in-charge and voucher specimens (GUD 152, GUD 298 & GUD 322) were deposited at the herbarium of the Department of Biology, The Gandhigram Rural Institute (Deemed to be University), Gandhigram, Tamil Nadu (India).

Extraction of the essential oil

The fresh leaves and swollen part of petioles of the plant were used to extract the essential oil through hydro-distillation using a Clevenger type apparatus for 4-6 h. The obtained essential oils were collected separately and dehydrated over anhydrous sodium sulfate and stored at 4 °C, under dark condition before analysis. The yield of the essential oil was calculated and expressed as the weight of the oil divided by the weight of the sample on a moisture-free basis (% v/w).

Gas chromatography and mass spectrometry (GC-MS) analysis

GC-MS analysis of extracted essential oils was performed by using a Shimadzu GC-MS QP2010 Ultra model and gas chromatograph interfaced to a mass spectrometer equipped with a Rxi-5Sil MS, fused silica capillary column (30 mL × 0.25 mm ID × 1 × df, composed of 100% dimethyl polysiloxane1,4-bis (dimethylsiloxy) phenylene dimethyl polysiloxane). For GC-MS detection, an electron ionization system with ionizing energy of 70 eV was used. Helium gas (99.999%) was used as the carrier gas at a constant flow rate 1 mL/min and an injection volume of 1 µL was employed (Split ratio of 50:1) injector temperature 250 °C; ion-source temperature 280 °C. The oven temperature was programmed from 100 °C (isothermal for 2 min) with an increase of 5 °C/min to 200 °C, then 10 °C/min to 280 °C, ending with a 2 min isothermal at 280 °C. Mass spectra were taken at 70eV; a scan-interval of 0.3 seconds and fragments from 40 to 800 Da. Total GC running time was 32 minutes and the software used to handle mass spectra and chromatograms was Lab Solutions. The compounds present in the oil of *T. zeylanicus* ssp. *travancoricus* were identified based on retention time and their identity was confirmed by comparing the mass spectra with the database from the Library of National Institute of Standard and Technology 11 (NIST 11) and Wiley8. Relative retention indices (RRI) of constituents of the essential oils were calculated concerning standards of straight-chain alkanes (C_7 to C_{33}- Restek Custom Retention Time Index Standard (Lot No: A0107972).

Antimicrobial activity of essential oil

Microorganisms tested

For the evaluation of the antimicrobial activity of the essential oils, five different microorganisms were procured from existing culture collections of the Department of Biology, The Gandhigram Rural Institute and the activity was tested against three Gram-negative bacteria (*Escherichia coli* ATCC25922, *Pseudomonas aeruginosa* ATCC27853, *Klebsiella pneumoniae* ATCC10031) and two Gram-positive bacteria (*Staphylococcus aureus* ATCC25923, *Bacillus subtilis* ATCC21332).

Disc diffusion assay

The antimicrobial activity of the essential oil samples was evaluated through the disc diffusion method. The selected microorganisms were separately cultured on nutrient agar plates for 24 h. Each test microorganism was sub-cultured on 10 mL nutrient broth and inoculated at 37±1 °C for 24 h. Then 100 µL of inoculums (10^6 cells/mL) were spread on the solid media plates. Filter paper discs (6 mm in diameter) were individually impregnated with 1 mL (5 and 10 µg) of the essential oil placed on the previously inoculated agar plates and the cultures were incubated at 37 °C for 24 h. Gentamicin (20 µg/disk) was used as the positive control and DMSO served as the negative control. The tests were carried out in triplicates and the diameter of the inhibition zone (DIZ) was measured in millimetres for each of the organisms and expressed as mean±S.E.

DPPH radical scavenging activity

The 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging capacity of essential oils was assessed as described by Blois[15] with slight modifications. A 1 mL aliquot of a methanolic solution of DPPH (0.1 mM) was added to 100 µL of essential oil solutions at different concentrations (10, 20, 30, 40, and 50 g/mL). The mixture was shaken vigorously and incubated for 30 minutes in the dark at 37 °C. Ascorbic acid was served as standard and subjected to the similar procedure to analyze the scavenging capacity. The absorbance was read against blank at 517 nm. Inhibition free radical DPPH in per cent was calculated by the following equation:

$$\text{Inhibition \%} = [(A_0 - A_s)/A_0] \times 100$$

where A_0 is the absorbance value of the blank (methanol) and A_s is the absorbance value of the sample. The concentration of essential oil providing 50% inhibition (IC_{50}) was calculated from the linear regression algorithm of the graph plotted. Tests were carried out in triplicates and values were expressed as mean±S.D.

Total antioxidant capacity by phosphomolybdenum assay

The total antioxidant activity of the essential oils was measured by the green phosphomolybdenum complex formation according to the method of Prieto *et al.*[16]. The single concentration (20 µg) of the sample was added to 1 mL of reagent solution (0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate) and incubated in a water bath at 95 °C for 90 min. After that, the samples were cooled to room temperature and the absorbance was measured at 695 nm against a blank. The absorbance of the reaction mixture indicated the increased reducing power of molybdenum. Ascorbic acid was used as standard and the total antioxidant activity of the essential oils was expressed as mg equivalent of AAE/g of essential oils. Triplicates were analyzed and the total antioxidant content was expressed as mean±S.E.

Results

Chemical composition of the essential oils

The chemical compositions of the essential oils of *T. zeylanicus* ssp. *travancoricus* were analyzed by GC-MS (Table 1). The fresh leaves gave aromatic pale green oil with 1.25% (v/w) yield. Through GC-MS, a total of sixteen compounds were detected, which accounts for 100%. Sesquiterpenes were the predominant group of the leaf oil, representing 90.99% of the total oil composition. The remaining compounds were reported as alcohol and ester which accounted for 4.8 and 4.19% respectively. Of the sixteen compounds detected, α-humulene (48.99%) and β-caryophyllene (30.08%) were the main

Table 1 — Chemical composition of essential oil of leaves and swollen part of petiole

S. No.	Compounds	RI	% GC		Method of identification
			Leaf	Petiole	
1	Linalool	1082	0.73	---	RI, MS
2	β-Caryophyllene	1494	30.08	11.96	RI, MS
3	Alloaromadendrene	1386	0.73	---	RI, MS
4	1-Heptatriacotanol	3942	0.76	---	RI, MS
5	α-Humulene	1579	48.99	36.69	RI, MS
6	Alloaromadendrene	1386	0.79	---	RI, MS
7	Cis-sesquisabinene hydrate	1523	1.07	---	RI, MS
8	β-citrylideneethanol	1465	1.11	---	RI, MS
9	α-Farnesene	1458	1.14	6.45	RI, MS
10	Nerolidyl acetate	1754	4.19	---	RI, MS
11	Caryophyllene oxide	1507	2.33	9.26	RI, MS
12	(+)- Ledene	1419	1.29	---	RI, MS
13	β-Guaiene	1523	1.99	3.62	RI, MS
14	(-)-Isolongifolol	1507	1.51	---	RI, MS
15	Nerolidol	1564	1.07	---	RI, MS
16	Phytol	2045	2.20	---	RI, MS
17	Selina-3,7(11)-diene	1523	---	6.92	RI, MS
18	Hexadecanoic acid, methyl ester	4765	---	17.41	RI, MS
19	Phthalic acid dioctyl ester	2832	---	2.47	RI, MS
20	Unknown	897	---	3.17	RI
21	Unknown	898	----	2.06	RI

components accounting for 79.07%. About 20.91% composed of trace components such as nerolidyl acetate (4.19%), caryophyllene oxide (2.33%), phytol (2.2%), β-guaiene (1.99%), alloaromadendrene (1.52%), (-)-isolongifolol (1.51%), (+)- ledene (1.29%), α-farnesene (1.14%), β-citrylideneethanol (1.11%), cis-sesquisabinene hydrate (1.07%), nerolidol (1.07%), linalool (0.73%), and 1-heptatriacotanol (0.76%) (Fig. 1-2).

The swollen part of the petiole yielded about 0.41% (v/w) of essential oil with higher consistency. About ten compounds were detected by GC-MS. Of these, eight compounds were identified and two were not identified which accounted for 94.78 and 5.23% respectively. The most abundant group of oil was sesquiterpenes, which is presented as 90.99% and the scarce compound was the palmitic acid group and phthalate group (4.8 and 4.19%). The major components of the total oil of the swollen part of petiole were recorded as α-humulene (36.69%), n-hexadecanoic acid (17.41%) and β-carophyllene (11.96%). However, caryophyllene oxide (9.26%), selina-3,7(11)-diene (6.92%), farnesane (6.45%), α-guaiene (3.62%), and phthalic acid dioctyl ester (2.47%) were detected as minor components of the total oil (Fig. 3).

Antimicrobial activity

Antimicrobial activities of the essential oils were determined by the agar disc diffusion method against five food-borne microorganisms and opportunistic

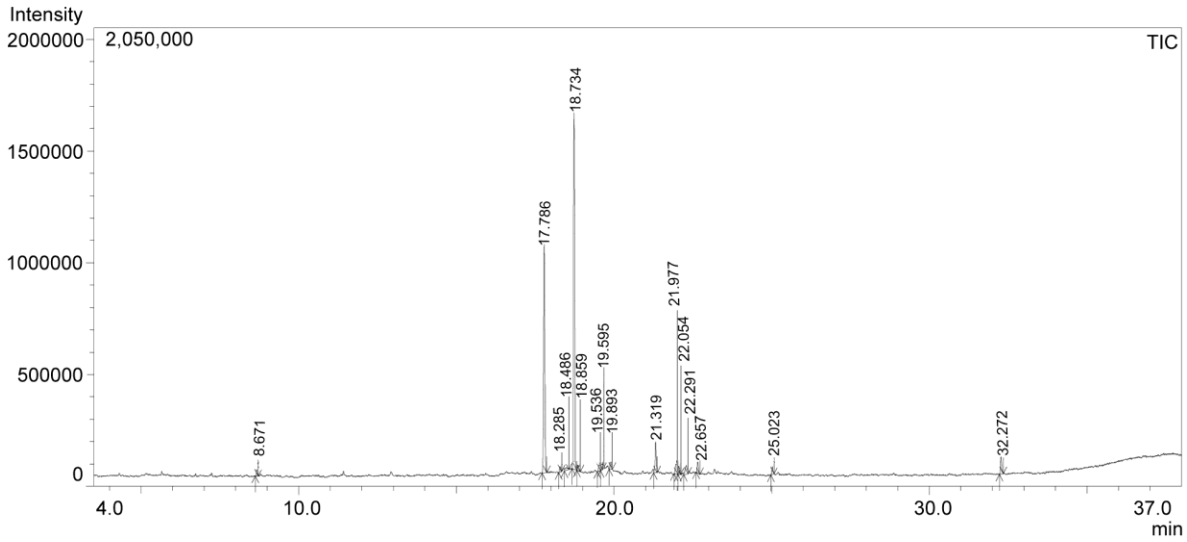

Fig. 1 — GC-MS Chromatogram of essential oil obtained from fresh leaves of *Trichopus zeylanicus* ssp. *Travancoricus*.

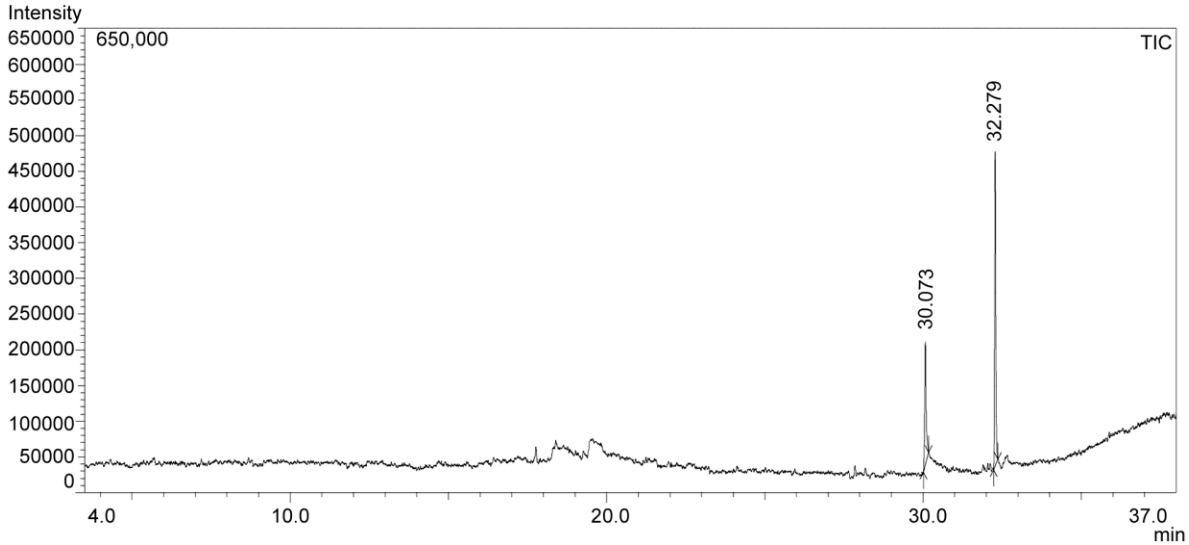

Fig. 2 — GC-MS Chromatogram of essential oil obtained from dried leaves of *Trichopus zeylanicus* ssp. *travancoricus*.

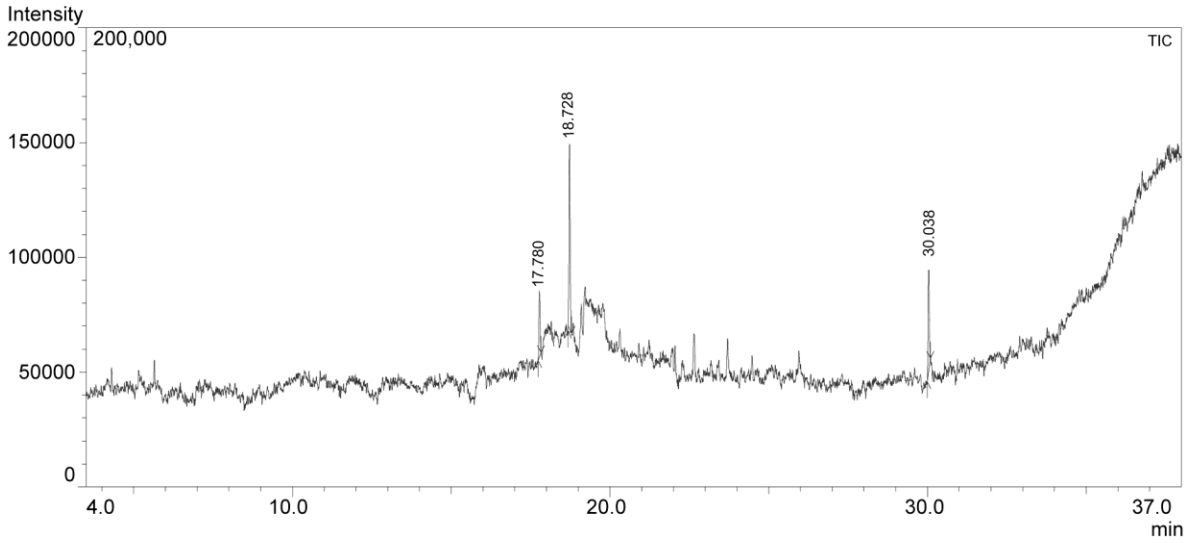

Fig. 3 — GC-MS Chromatogram of essential oil obtained from petiole of *Trichopus zeylanicus* ssp. *Travancoricus*

pathogens. The essential oils showed moderate to better activity against all the tested microorganisms (Table 2). At 10 µg concentration, both essential oils exhibited strong inhibition activity against *P. aeruginosa* and the comparable zone of inhibition was noted in the leaf essential oil (20.33±0.88mm) and swollen part of the petiole (19.66±0.88 mm). Moreover, the oils exhibited moderate activity against *K. pneumoniae*, *E. coli*, and *S. aureus* with a diameter of inhibition zone ranging from 13.6 to 15.6 mm.

Antioxidant activity

The free radical scavenging capacities of the oils were evaluated by DPPH assay and the results are reported in Table 3. The results indicated that the radical-scavenging activity of both the essential oils (leaf and petiole) and positive control were dose-dependent. The oils were reported to possess poor DPPH radical scavenging activity with IC_{50} value of more than 1000 µg/mL. Moreover, the total antioxidant capacities (TAC) of the essential oils were 1.85±0.03 and 1.70±0.01 mg ascorbic acid equivalent AAE/g in the leaf and petiole respectively.

Table 2 — Antibacterial activity of essential oils of *T. zeylanicus* ssp. *travancoricus*

Microorganisms	Leaves (mm)	Swollen part of petiole (mm)	Gentamicin (mm)
Escherichia coli	15.66±0.33	14.00±0.57	13.66±0.33
Klebsiella pneumoniae	15.33±0.33	13..66±0.33	28.33±0.33
Pseudomonas aeruginosa	20.33±0.88	19.66±0.88	11.66±0.88
Staphylococcus aureus	14.00±0.7	14.66±0.88	26.33±0.57
Bacillus subtilis	13.33±0.88	12.00±0.57	23.33±0.33

Table 3 — Antioxidant activity of essential oils of *T. zeylanicus* ssp. *travancoricus*

Samples	DPPH (IC_{50} (µg/mL))	TAC mg AAE/g of oil
Ascorbic acid	14.07±0.97	---
Leaf	>1200	1.70±0.01
Swollen part of petiole	>1000	1.85±0.03

Discussion

Usually, terpenes and terpenoids are the prime groups of the essential oils of plants and the remaining constituents consist of aromatic and aliphatic compounds[17-19]. Terpenes were the major compounds of the essential oils of leaves and swollen part of the petioles of *T. zeylanicus* ssp. *travancoricus*. Moreover, sesquiterpene was the predominant group of essential oils accounting for 90.99% of the total oil. Sesquiterpenes have been reported as a major component of Chinese ginseng[20]. Moreover, humulene and caryophyllene have resulted from C1-C10 cyclization of farnesyl diphosphate. α-Humulene, a monocyclic sesquiterpene which has 11-membered ring consists of three isoprene units containing three nonconjugated C═C double bonds. In the present study, α-Humulene and β-Caryophyllene were reported as major compounds. However, similar result was noticed from the essential oil of *Eleutherococcus senticosus* (Siberian ginseng) with rich in β-caryophyllene (21.7%) and α-humulene (7.4%) as principal constituents[21]. These compounds

were also noticed from the essential oils of *Humulus lupulus*[22] and *Cannabis sativa*[23]. α-Humulene has act as an anorectic (appetite suppressant) and usually blended with β-caryophyllene and reported to have diversified biological activities, anti-inflammatory, analgesic, and anti-cancer activities[24]. The current phytochemical analysis of the essential oil of leaves confirmed the previous ethnobotanical report[25]. β-Caryophyllene was reported as the most abundant active phytocompound represented from the essential oil of fresh leaves and swollen part of the petiole of *T. zeylanicus* ssp. *travancoricus*. Generally, β-caryophyllene (bicyclic sesquiterpene) is the only sesquiterpene that interacts with the endocannabinoid system (ECS) of the body and maintains homeostasis in organisms which regulate stable energy and hormone levels, neurotransmitter concentrations, temperature, and more. Even, if the organism is not in perfect homeostasis, there will be a possibility of diseases in the body[26]. Nerolidol (1.07%) is sesquiterpene alcohol which has multifaceted pharmacological and biological activities[27]. Linalool was reported as a minor compound (0.73%) of the fresh leaf oil and it has an effective anxiety and stress reliever property[28]. Besides, α-farnesene (1.14%) and β-guaiene (1.99) have also been reported as minor compounds commonly used in the fragrance industry[29-31]. Phytol is a diterpene alcohol which occupies 2.2% of the total essential oil isolated from fresh leaves. Usually, it is formed due to the degradation of chlorophyll, which inhibits the enzyme that degrades the neurotransmitter GABA and may partially account for its relaxing effect[32]. Further, phytol can be used to enhance energy, to fight against infections and also used as natural alternatives for hypertension and cancer[33]. In this study, hexadecanoic acid, methyl ester occupied 17.41% of the total oil of petiole. The compound has exhibited antidiabetic activities[34]. Furthermore, hexadecanoic acid methyl ester also has alpha-reductase inhibitor activities[35,36]. A significant amount of hexadecanoic acid methyl ester has been reported earlier from the essential oil of *Russelia equsetiformis*[37].

Antimicrobial activity

The antimicrobial properties of essential oils of *T. zeylanicus* ssp. *travancoricus* were evaluated using disc diffusion method. To-date, no reports are available about the antimicrobial activity of essential oil of *T. zeylanicus* ssp. *travancoricus*. The essential oil possesses a number of components and each compound exhibits its unique mechanism of action on organisms. On the whole, the mechanism of antibacterial action is carried out by a series of biochemical reactions in the bacterial cell which mainly depend on the chemical constituents[36,37]. Based on the chemical constituents and the quantity of major compounds of the essential oil, the efficiency of antimicrobial activity is determined[37]. Moreover, the antimicrobial activity of essential oil has also exhibit differences in sensitivity between the Gram-negative and Gram-positive bacteria[38,39]. Comparatively, Gram-negative bacteria are less susceptible than Gram-positive bacteria. The outer membrane of Gram-negative bacteria endowed with strong hydrophilicity act as a strong permeability barrier[40]. But, in this study, the essential oils of both leaves and swollen parts of petiole showed maximum zone of inhibition against *P. aeruginosa*. The lipophilic compounds of the oils interact with the phospholipid bilayer of the cell membrane of the organism and increase its permeability and spread out the intracellular contents, thereby damaging the enzymatic system of the cell[41]. The changes occurring in cytoplasmic membrane affects the metabolism and macromolecule synthesis[42]. *Pseudomonas aeruginosa*, a multidrug-resistant bacterium has been reported with highest susceptibility against both essential oils than the antibiotic (gentamycin). The present study is in agreement with a previous report[43]. The present study suggest that the antimicrobial activity of the essential oil of *T. zeylanicus* ssp. *travancoricus* against *P. aeruginosa* might be due to the higher percentage of α-humulene and β-caryophyllene. The major chemical constituents reported from the essential oil were humulene, β-caryophyllene, hexadecanoic acid methyl ester, β-caryophyllene oxide, nerolidyl acetate, phytol, selina-3,7(11)-diene, farnesane, α-guaiene, and phthalic acid dioctyl ester. These compounds were found to be equally effective against both Gram-positive and Gram-negative organisms. The present result corroborated with previous reports[43-46].

DPPH radical scavenging assay

Essential oils of leaf and petiole exhibited poor to moderate antioxidant activity, since, both oils were rich in sesquiterpenes. Moreover, the terpenes are not able to donate a hydrogen atom and exhibit low solubility in the reaction mixture of radical scavenging assay[47]. So, both essential oils gave higher IC_{50} value than standard. Even though the higher

antioxidant potential was noticed in the petiole oil than leaf oil due to the presence of hexadecanoic acid methyl ester and this is supported by previous literature[48,49].

Total antioxidant capacity

Phosphomolybdenum assay is used to estimate the compound of both hydrophilic and lipophilic soluble antioxidant capacity. The antioxidant activity is mainly focused on redox properties of compounds, which provides their activity as hydrogen donors, reducing agents, singlet oxygen quenchers, metal chelators and reductants of ferryl haemoglobin. The reducing ability is invariably related to the presence of reductants that provide antioxidant action by dissociating the free radical chain by donating a hydrogen atom[50]. The assay results indicated that the essential oil has an antioxidant capacity. Comparatively, the swollen part of petiole exhibited a higher total antioxidant quantity than the leaf. The swollen part of petiole oil has hexadecanoic acid as a second major component.

Conclusion

Antimicrobial and antioxidant properties of the essential oils possess a great interest in food and pharmaceutical industries and also as the natural alternative sources for synthetic preservatives. The essential oils of leaves and petiole are reported with promising phytocompounds that have the potential to cure various ailments. The study concludes that they have strong antimicrobial activity and mild antioxidant activity. This study is an experimental proof of the previous attempts on ethnobotanical investigations carried out among Kani tribes of Agasthymalai Biosphere Reserve. The essential oil of this potential herb can be considered as an alternative medicine to treat various diseases and may used in the food and pharmaceutical industries.

Conflict of interest

Authors declare no competing interest.

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