

# Investigation of Antioxidant and Antibacterial Properties of Leaf, Root, Fruit and Stem Extracts of *Trichopus zeylanicus* from South India

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## ABSTRACT

Objective: To investigate the total phenols, flavonoids and carotenoids content, antioxidant and antibacterial potential profile of leaf, root, fruit and stem extracts of the *Trichopus zeylanicus* with five major solvents, that is, water, methanol, chloroform, ethyl acetate, and petroleum ether. Results: The total phenols, flavonoids and carotenoids contents of fruit methanol extract of *T.zeylanicus* was found to be 85.15±0.223 mg catechol equivalent per gram, 165.5622±1.236 mg quercetin equivalent per gram and 84.47273 ±0.531 mg β-Carotene equivalent per gram respectively which are higher than the activity of other extracts. The methanol extracts showed more reducing power, metal chelating activity, hydrogen peroxide scavenging and phosphomolybdenum assays than other extracts and their activity increased with increasing concentration of extracts. The hydroxyl radical scavenging activity of fruit methanol extract is quite comparable to the standard mannitol of the assay and its antibacterial potential is the best way to cope with infectious action of bacteria. Conclusion: The remarkable activity showed by the plant extracts could be attributed to the synergic effect of the active compounds present in it. Among the extracts, methanol was found to be the better solvent to extract natural products to get maximum medicinal benefits.

**Keywords:** *Trichopus zeylanicus*, antioxidants, antibacterial activity, phenolics, flavonoids, carotenoids.

## INTRODUCTION

Oxidation reactions lead to production of reactive oxygen species mainly free radicals which in turn start chain reactions causing damage to DNA, proteins and lipids, which have been associated with carcinogenesis, coronary heart diseases and many other health problems<sup>1</sup>. Antioxidants obstruct the chain reactions by scavenging these free radical intermediates thus preventing such diseases. Plants are the primary sources of naturally occurring antioxidants for humans. Natural antioxidants are danger-free and cause fewer adverse health impacts than synthetic antioxidants<sup>2</sup>. Therefore, considerable interests in development of natural antioxidants have received much highlight in recent years, especially within biological, medical, nutritional and in agrochemical areas<sup>3</sup>. Treatment of infectious diseases is a big challenge due to multi-drug resistance, as pathogens gets resistance to existing antibiotics. It is therefore highly desirable to explore plants for new alternative antimicrobial agents to treat infectious diseases. The present investigation deals with the determination of antioxidant and antibacterial capability of leaf, root, fruit and stem extracts of *Trichopus zeylanicus*.

*Trichopus zeylanicus* Gaertn. (Trichopodaceae) is a rare, herbaceous perennial and rhizomatous plant growing along the tropical forests in southern India, Malaysia and Sri Lanka. *Kani*, a tribe living in high altitude places in southern India, use the seeds of *Trichopus zeylanicus* to

fight against fatigue<sup>2</sup>. The tribal inhabitants of this area (*Kani* tribes) are using this plant for replenishing the stamina<sup>4</sup>. Further studies using a laboratory animal model confirmed the anti-fatigue properties of the plant in the forced swim test. Administration of ethanolic leaf extract to male mice stimulated their sexual behavior as evidenced by an increase in number of mounts and mating performance<sup>5</sup>. The alcoholic extract of seeds of *Trichopus zeylanicus* showed adaptogenic<sup>6</sup> or anti-stress in both rats and mice. This extract also enhances swimming performance in mice<sup>7</sup>. Oral administration of *Trichopus zeylanicus* induces immunomodulation that protect mice from tumour cell growth<sup>8</sup>. *Trichopus zeylanicus* extracts have been evaluated for its anti hepatoprotective and choleric activities in rats<sup>6</sup>. A glyco-peptido lipid fraction from the alcoholic extract of the herb was evaluated for anti stress activity<sup>7</sup>. Adaptogenic activity was showed by the glyco peptido-liquid fraction from the alcoholic extract of *Trichopus zeylanicus*<sup>8</sup>. The leaves of *Trichopus zeylanicus* is used by *Kannikars* for scabies and ring worm infections<sup>9</sup>, animal studies showed the cardio protective effect of the plant<sup>10</sup>, and anti-cancer activity of a herbal composition of this plant<sup>11</sup>.

There are greater number of pharmacological potential activities reported on this plant and combining the tribal knowledge and the modern pharmacological works, the present work is to investigate the presence of secondary metabolites in the extracts of the plant, their antioxidant

and antibacterial abilities through reliable methods. The results showed that the methanol extract of leaf, root, fruit and stem of *Trichopus zeylanicus* are good source of antioxidant and antibacterial potential agents<sup>12</sup>.

## MATERIALS AND METHODS

### Chemicals

Sodium phosphate, potassium ferricyanide, ammonium molybdate, quercetin, ascorbic acid, aluminium chloride, potassium hydroxide, potassium acetate, trichloro acetic acid, ferric chloride, ferrozine, EDTA, diethyl ether, and catechol, mannitol,  $\beta$ -carotene, 2-thiobarbituric acid (TBA), butylated hydroxyl anisole (BHA), Folin-Ciocalteu, 2- deoxyribose and  $H_2O_2$ (30 %). All other chemicals and solvents used were of analytical grade.

### Test microorganisms

The microorganisms used for antibacterial activity evaluation were obtained from Microbial Type Culture Collection and gene bank (IMTECH, Chandigarh, India), which were maintained on Nutrient broth media. They were Gram-positive bacteria such as *Bacillus cereus* (MTCC-1305) and *Staphylococcus aureus*(MTCC-96) and Gram-negative bacteria such as *Escherichia coli* (MTCC-729), *Klebsiella pneumoniae*(MTCC-109), *Pseudomonas aeruginosa*(MTCC-647) and *Proteus vulgaris* (MTCC-426).

### Culture medium and inoculum

The stock cultures of microorganisms used in this study were maintained on Plate Count Agar slants at 4°C. Inoculum was prepared by suspending a loop full of bacterial cultures into 10 mL of nutrient broth and was incubated at 37°C for 24 hours. On the next day Muller-Hinton agar (MHA) (Merck) sterilized in a flask and cooled to 45-50°C was distributed by pipette (20 mL) into each sterile Petri dish and swirled to distribute the medium homogeneously. About 0.1 mL of bacterial suspension was taken and poured into Petri plates containing 20 mL nutrient agar medium. Using the L-shaped sterile glass spreader bacterial suspensions were spread to get a uniform lawn culture.

### Sample collection

Five hundred small herbs of *Trichopus zeylanicus* (600g) were collected from Agasthyar hills of Kerala in the month of October 2012 through a government approved agency Rayirath Gardens – Pattikkad Thrissur, Kerala and authenticated by Dr. Kochuthressia M.V., HOD, Department of Botany, Vimala College, Thrissur. Voucher specimen is deposited in the specially maintained herbarium, Department of Botany, Vimala College, Thrissur, Kerala.

### Preparation of Extracts

The air dried and powdered leaves, root, fruits and stem of plant material were separately extracted successively with 150ml of petroleum ether, chloroform, ethyl acetate, methanol and water as solvents for 24hours by Soxhlet equipment.

### Determination of total phenolics

Folin-Ciocalteu (FC) assay was used to the determination of the total phenolics (TP) content of the leaf, root, fruit and stem extracts of *Trichopus zeylanicus*. Eight ml of

water was added into 1 ml of extract in a 10 ml volumetric flask. 0.5 ml of FC reagent was added and mixed for 15 min followed by addition of 1.5 ml of 20 % sodium carbonate solution. After 2 h at ambient temperature the absorbance of the colored reaction product was measured at 765 nm, where different concentrations of standard catechol solutions were used for calibration curve and results were expressed as mg of catechol Equivalent per gram (mg catechol/g) of dried extract (standard plot:  $y = 0.0966x$ ,  $R^2 = 0.9878$ )<sup>13</sup>.

### Determination of total flavonoids

The total flavonoid content of leaf, root, fruit and stem extracts of *Trichopus zeylanicus* were determined by using aluminium chloride colorimetric method<sup>14</sup>. Quercetin was used as a standard to make the calibration curve. The sample solution (0.5 ml) was mixed with 1.5 ml of 95 % ethanol, 0.1 ml of 10 % aluminium chloride hexahydrate, 0.1 ml of 1 M potassium acetate, and 2.8 ml of distilled water. After incubation at room temperature for 40 min the absorbance of the reaction mixture was measured at 415 nm. The same amount (0.1 ml) of distilled water substituted for the amount of 10 % aluminum chloride as the blank and a seven point standard curve (0-500  $\mu$ g/ml) ( $y = 0.0148x$ ,  $R^2 = 0.975$ ), was obtained<sup>15</sup>.

### Determination of carotenoids

Total carotenoids were determined<sup>16</sup> by extracting one gram of sample with 100 ml of 80 % methanol solution and centrifuged at 4 000 rpm for 30 min. The supernatant was concentrated to dryness. The residue was dissolved in 15 ml of diethyl ether and after addition of 15 ml of 10 % methanolic KOH the mixture was washed with 5 % ice-cold saline water to remove alkali. The free ether extract was dried over anhydrous sodium sulphate for 2 h. The ether extracts were filtered and its absorbance was measured at 450 nm by using ether as blank.  $\beta$ -carotene ( $y = 0.022x - 0.008$ ,  $R^2 = 0.997$ ) has taken as standard.

### Antioxidant Activity

#### Reducing power assay

Aliquots of each extracts were taken in test tubes and dissolved in 1 ml of 0.2 M phosphate buffer in a test tube to which was added 5 ml of 0.1 % solution of potassium ferric cyanide<sup>17</sup>. The mixture was incubated 50°C for 20 min. Following this, 5 ml of trichloroacetic acid (10 %) (w/v) solution was added and the mixture was then centrifuged at 7000 rpm for 10 min. A 5 ml of aliquot of the upper layer was combined with 5 ml of distilled water and 1 ml of ferric chloride solution (0.1%) and absorbance was recorded at 700 nm against reagent blank. A higher absorbance of the reaction mixture indicates greater reducing power of the sample.

#### Metal chelating activity

The chelating of ferrous ions by *T.zylanicus* extracts were estimated<sup>18</sup> by adding extract samples (250  $\mu$ l) to a solution of 2 mmol/l  $FeCl_2$  (0.05 ml). The reaction was initiated by the addition of 5mmol/l ferrozine (0.2 ml) and the mixture was shaken vigorously and left standing at room temperature for 10 min, after which the absorbance was measured spectrophotometrically at 562 nm. The chelating activity of the extracts was evaluated using EDTA as

standard. The metal chelating activity of the extract is expressed as mg EDTA equivalent/g extract.

#### Phosphomolybdenum activity

The antioxidant activity of samples was evaluated by the green phosphomolybdenum complex formation method<sup>19</sup>. An aliquot of 100 µL of sample solution was combined with 1 ml of reagent solution (0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate) in a 4 ml vial. The vials were capped and incubated in a water bath at 95°C for 90 min. After the samples have cooled to room temperature, the absorbance of the mixture was measured at 695 nm against a blank. The results reported (Ascorbic acid equivalent antioxidant activity) are mean values expressed as g of ascorbic acid equivalents/100g extract.

#### Hydrogen peroxide radical scavenging activity

Hydrogen peroxide assay<sup>20</sup> was carried out for the determination of antioxidant activity of compounds for their ability to scavenge the oxidant hydrogen peroxide. The reaction mixture contained phosphate buffer (pH-7.4) and hydrogen peroxide solution prepared in phosphate buffer (40 mM). Plant extracts at the concentration of 10 mg/10µL was added to hydrogen peroxide solution (0.6 ml, 40 mM). The total volume was made up to 3 ml. The absorbance of the reaction mixture was recorded at 230 nm. The blank solution contained phosphate buffer without hydrogen peroxide. The percentage of hydrogen peroxide scavenged by the plant extract was calculated as follows:  
Percentage of scavenged H<sub>2</sub>O<sub>2</sub> =  $\frac{A_0 - A_{\text{sample}}}{A_0} \times 100$

Where, A<sub>0</sub>-Absorbance of control  
A<sub>sample</sub> - Absorbance in the presence of plant extract.

#### Hydroxyl radical scavenging activity

Hydroxyl radicals were generated by a Fenton reaction (Fe<sup>3+</sup>-ascorbate-EDTA-H<sub>2</sub>O<sub>2</sub> system), and the scavenging capacity towards the hydroxyl radicals was measured by using deoxyribose method<sup>21</sup>. The reaction mixture contained 2-deoxy-2-ribose (2.8 mM), phosphate buffer (0.1 mM, pH 7.4), ferric chloride (20 µM), EDTA (100 µM), hydrogen peroxide (500 µM), ascorbic acid (100 µM) and various concentrations (10-1000 µg/ml) of the test sample in a final volume of 1 ml. The mixture was incubated for 1 h at 37 °C. After the incubation an aliquot of the reaction mixture (0.8 ml) was added to 2.8% TCA solution (1.5 ml), followed by TBA solution (1% in 50 mM sodium hydroxide, 1 ml) and sodium dodecyl sulphate (0.2ml). The mixture was then heated (20 min at 90 °C) to develop the colour. After cooling, the absorbance was measured at 532 nm against an appropriate blank solution. All experiments were performed in triplicates. The percentage of inhibition was expressed, according to the following equation:

% Inhibition =  $\frac{[A_0 - (A_1 - A_2)]}{A_0} \times 100$ , where, A<sub>0</sub> is the absorbance of the control without a sample, A<sub>1</sub> is the absorbance in the presence of the sample and deoxyribose and A<sub>2</sub> is the absorbance of the sample without deoxyribose.

#### Antibacterial activity assay

The agar well diffusion method is used for the antimicrobial evaluations. Wells of 8mm (0.8cm) diameter were dug on the inoculated nutrient agar medium with sterile cork borer and 50 µL of the petroleum ether, ethyl acetate, chloroform, methanol and water extracts of the leaves, root, fruit and stem of *T.zylanicus* were added in each well. Wells introduced with 50 µL of pure petroleum ether, ethyl acetate, chloroform methanol and water served as negative controls. The plates were incubated at 37°C over night and examined for the zone of inhibition. The diameter of the inhibition zone was measured in mm. The standard antibiotic drugs such as streptomycin and penicillin were also screened under similar conditions for comparison. An extract was classified as active when the diameter of the inhibition was equal to or larger than 8mm. All the assays were performed in triplicate and mean values were presented<sup>22</sup>.

#### Statistical analysis

Results were expressed as the means of three replicates ± the standard deviation of triplicate analysis.

## RESULTS AND DISCUSSIONS

#### Determination of total phenols, flavonoids and carotenoids

Total phenolic content of the leaf, root, fruit and stem methanol extracts of *T.zylanicus* respectively are 61.822±0.336, 20.849 ±0.5713, 85.15±0.223 and 21.47±0.187 mg catechol equivalent per gram of plant extract . Among these, the fruit methanol plant extract contains more phenolic component than other extracts. The phenolic compounds present in natural products have higher antioxidant activity and can act as free radical scavengers<sup>23</sup>.

The flavonoid contents of the *T.zylanicus* fruit methanol extract are (165.5622 ±1.236) mg Quercetin equivalent per gram of plant extract which is higher than *T.zylanicus* leaf (157.264 ±2.102), root (150.0135 ±1.582) and stem (142.5135 ±1.850) methanol extracts . The flavonoids possess antioxidant activity, acting through scavenging or chelating process thereby having significant effect on human health<sup>24</sup>. Since the fruit methanol extract of *T.zylanicus* found to have higher amount of flavonoid compounds, suggesting their usage as a good source of natural antioxidant, preventing free radical-mediated oxidative damage.

The total carotenoid contents of the *T.zylanicus* fruit methanol extract is (84.47273 ±0.531) mg β-Carotene equivalent per gram of plant extract. The total carotenoid contents of the *T.zylanicus* leaf (77.41364 ±0.236), root (61.16364 ±0.581), stem (65.33182 ±1.238) methanol extracts are lower than total carotenoid contents of *T.zylanicus* fruit methanol extracts.

#### Reducing power assay

The reducing power of the petroleum ether, ethyl acetate, chloroform, methanol and aqueous extracts of leaf, root, fruit and stem of *Trichopus zylanicus* using potassium ferricyanide reduction method. The absorbance value of the extract shows higher increase with increase in concentration and methanol extracts show more activity than the activity of standard L ascorbic acid. The yellow

Table 1: Metal chelating activity of *Trichopus zeylanicus* leaf extracts

Samples	IC <sub>50</sub> values of metal chelating activity of					
	Petroleum Extracts	Ethyl acetate Extracts	Chloroform Extracts	Methanol Extracts	Aqueous Extracts	
TZ Leaf Extracts	124.38 ± 0.065	95.6 ± 0.031	63.05 ± 0.02	59.55 ± 0.03	70.21 ± 0.04	
TZ Root Extracts	91.51 ± 0.85	52.28 ± 0.26	52.66 ± 0.52	46.92 ± 0.22	62.27 ± 0.43	
TZ Fruit Extracts	91.07 ± 1.05	44.44 ± 0.53	50.94 ± 0.09	41.36 ± 0.089	59.67 ± 0.52	
TZ Stem Extracts	99.13 ± 0.98	83.995 ± 0.65	69.67 ± 0.38	60.42 ± 0.27	67.93 ± 0.86	
EDTA (standard) IC <sub>50</sub> value- 39.49 ± 0.22						

Table 2: Phosphomolybdenum activity of *Trichopus zeylanicus*.

Sample	Antioxidant potential content (mg L Ascorbic acid equivalent/100g Plant extract)
TZ leaf Methanol extract	187.91 ± 0.256
TZ Root Methanol extract	144.5923 ± 1.62
TZ Fruit Methanol extract	192.056 ± 0.82
TZ Stem Methanol extract	108.8278 ± 0.109

colour of test solution changes to various shades of green and blue due to the reduction of Fe<sup>3+</sup>/ Ferric cyanide complex to ferrous form by the antioxidants present in the extract. Thus, the reducing power of medicinal plants and vegetables are said to be well attributed with the antioxidant activity.

#### Metal chelating activity

The metal chelating activity of the petroleum ether, ethyl acetate, chloroform, methanol and aqueous extracts of leaf, root, fruit and stem of *Trichopus zeylanicus* are given in the tables 4. The IC<sub>50</sub> value of plant extracts (Table 1) were compared to the standard EDTA 39.49 µg/ml and it was found that the IC<sub>50</sub> values of the methanol extracts were close to the IC<sub>50</sub> value of EDTA. In this metal chelating activity, the presence of chelating agents in the extract of *T.zeylanicus* disrupts the ferrozine-Fe<sup>2+</sup> complex formation, thus decreasing the red colour. It was reported that chelating agents are effective as secondary antioxidants as they stabilize the oxidized form of the metal ion by reducing the redox potential.

#### Phosphomolybdenum activity

The total antioxidant activity of the methanol extracts of leaf, root, fruit and stem of *Trichopus zeylanicus* extract were determined by phosphomolybdenum method. Among these extracts the fruit methanol extract showed more activity and the higher value determines more antioxidant activity on this assay (table 2). This method is based on the formation of green phosphomolybdenum complex at 95 °C measured at an intensity of absorbance at 695 nm. In this method, reduction of Mo (VI) to Mo (V) by the antioxidant compounds present in the plant extract, forming green phosphate/Mo (V) complex takes place. Based on the before did antioxidant assay, the methanolic extract of leaf, root, fruit and stem of *Trichopus*

*zeylanicus* showed more significant activity than petroleum ether, ethyl acetate, chloroform and aqueous solvent extracts and the basis of selection of the methanol extract is this assay.

#### Hydrogen peroxide radical scavenging activity

The hydrogen peroxide radical scavenging activity of the methanol extracts of leaf, root, fruit and stem of *Trichopus zeylanicus*, showing the IC<sub>50</sub> value of 92.697±1.25, 107.63±0.656, 86.314±1.256 and 108.58±0.82 µg/ml compared with standard ascorbic acid (IC<sub>50</sub>=51.23±1.25 µg/ml). Hydrogen peroxide, though not reactive, is said to be highly important because of its ability to penetrate biological membranes, releasing toxic hydroxyl radicals in the cells. Thus the plant extracts showed significant scavenging activity of H<sub>2</sub>O<sub>2</sub>.

#### Hydroxyl radical scavenging activity

At a concentration of 1 mg/ml, the scavenging activity of fruit methanol extract of *T.zeylanicus* and standard mannitol were found to be 67% and 82.70% respectively. The IC<sub>50</sub> value of fruit methanol extract 51.5 ± 1.10 µg/ml was found to be quite comparable to the IC<sub>50</sub> value of standard mannitol 48.9 ± 2.03 µg/ml. Thus, fruit methanol extract is more effective in quenching the hydroxyl radicals produced in the reaction mixture. The *T.zeylanicus* extract compete with deoxyribose and diminish chromogen formation in a dose dependant manner. In this assay, 2-deoxy-2-ribose was oxidized when exposed to hydroxyl radicals generated by Fenton-type reaction. The oxidative degradation can be detected by heating the products with TBA under acid conditions to develop a pink chromogen (thiobarbituric acid reactive species). Among the leaf, root, fruit and stem extracts of various solvents, the methanol fruit extract showed more potential antioxidant activity.

#### Antibacterial screening of *T.zeylanicus* extracts

As can be seen from Table 8, the fruit extracts of *T.zeylanicus* showed pronounced antibacterial activity at concentration 250mg/ml against all the microorganisms tested. Among the extracts, methanol extract exhibited higher activity and petroleum ether extract showed least activity against all the tested organisms such as *Bacillus cereus*, *Staphylococcus aureus*, *Escherichia coli*, *Proteus vulgaris*, *Klebsiella pneumonia* and *Pseudomonas aeruginosa* (table 3).

## CONCLUSION

Table 3: Antibacterial activity of all major part extracts in aqueous and organic solvent *T zeylanicus* against gram positive and gram negative bacterial strains

Micro organisms	Inhibition zone in diameter (mm)					
	Streptomycin	Pencilin	Ethylacetate	Chloroform	Methanol	Water
<b>Leaf</b>						
<i>B cereus</i>	18.80± 0.85	19.36±0.47	15 ±0.51	16±1.02	17 ±0.45	9±0.43
<i>S aureus</i>	15.70± 0.65	14.50±0.35	12±0.92	13 ±0.50	14±0.30	5±0.61
<i>P aeruginosa</i>	13.10± 0.49	17.30±0.81	10±0.74	12 ±0.95	12 ±0.19	9±0.31
<i>E. coli</i>	14.70± 0.88	15.60±0.25	10 ±0.40	10±0.78	12 ±0.51	6±0.90
<i>K pneumonia</i>	20.10± 0.47	20.10±0.85	12±0.80	13±0.43	15 ±0.73	8±0.63
<i>P vulgaris</i>	19.80± 0.57	20.10±0.45	12±0.59	13±0.51	15 ±0.47	9±0.37
<b>Root</b>						
<i>B cereus</i>	18.80± 0.85	19.36±0.47	14±0.29	15 ±0.59	18 ±0.28	8±0.50
<i>S aureus</i>	15.70± 0.65	14.50±0.35	12 ±0.37	12 ±0.62	13±0.37	7±0.25
<i>P aeruginosa</i>	13.10± 0.49	17.30±0.81	12±0.62	14±0.32	15 ±0.47	8±0.51
<i>E. coli</i>	14.70± 0.88	15.60±0.25	9±1.98	10 ±0.71	13 ±0.42	7±0.41
<i>K pneumonia</i>	20.10± 0.47	20.10±0.85	15 ±0.76	15 ±0.81	20 ±0.72	8±0.63
<i>P vulgaris</i>	19.80± 0.57	20.10±0.45	8±0.84	10±0.31	12 ±0.80	8±0.45
<b>Fruit</b>						
<i>B cereus</i>	18.80± 0.85	19.36±0.47	15±0.83	16±0.72	19±0.36	10±0.51
<i>S aureus</i>	15.70± 0.65	14.50±0.35	12±0.99	14±0.82	15±0.61	9±0.31
<i>P aeruginosa</i>	13.10± 0.49	17.30±0.81	12±0.95	12±0.63	13 ±0.23	9±0.42
<i>E. coli</i>	14.70± 0.88	15.60±0.25	15 ±0.54	15±0.31	15 ±0.59	8±0.52
<i>K pneumonia</i>	20.10± 0.47	20.10±0.85	14±0.56	15 ±0.45	20±0.42	9±0.90
<i>P vulgaris</i>	19.80± 0.57	20.10±0.45	15 ±0.98	18±0.82	20±0.98	8±0.72
<b>Stem</b>						
<i>B cereus</i>	18.80± 0.85	19.36±0.47	14±0.39	12±0.45	16 ±0.56	7±0.53
<i>S aureus</i>	15.70± 0.65	14.50±0.35	11±0.72	12 ±0.40	12±0.61	6±0.81
<i>P aeruginosa</i>	13.10± 0.49	17.30±0.81	9±0.87	11±0.30	13±0.61	5±0.43
<i>E. coli</i>	14.70± 0.88	15.60±0.25	9±0.73	10 ±0.81	15 ±0.82	8±0.61
<i>K pneumonia</i>	20.10± 0.47	20.10±0.85	10±0.28	15 ±0.83	16 ±0.31	7±0.45
<i>P vulgaris</i>	19.80± 0.57	20.10±0.45	10±0.93	11±0.52	15±0.42	9±0.52

Antioxidants are resistance against the oxidative stress by scavenging free radicals and by other mechanism and prevent body from oxidative diseases. Plants are identified as the source of natural antioxidants that can protect body from oxidative decay and has reported less side effect. The present study indicates that the leaf, root, fruit and stem methanolic extracts of *Trichopus zeylanicus* are high in phenolic, flavonoid and carotenoid content. Flavonoids are potent antioxidants having characteristics of scavenging free radical, chelating metal and inhibiting lipid peroxidation. Out of petroleum ether, ethyl acetate, chloroform, methanol and aqueous extracts of leaf, root, fruit and stem of *Trichopus zeylanicus*, the methanol extract of leaf, root, fruit and stem possessed strong antioxidant activity. Also the results of reducing power assay, phosphomolybdenum activities, show that *T.zeylanicus* as promising natural sources of antioxidants suitable for preventing free radical-mediated reaction. The extracts exhibited high antibacterial activity against important bacterial strains. Among the five solvent extracts, methanol was found to be the best solvents of choice to extract natural products to get maximum medicinal benefits. Further studies are needed to explore the potential phenolics, flavonoid and carotenoid compounds from *T.zeylanicus* for application in drug delivery, nutritional or pharmaceutical fields.

#### CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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#### REFERENCES

1. Autore G, Caruso A, Marzocco S, Nicolaus B, Palladino C, Pinto A, Popolo A, Sinicropi MS, Tommonaro G and Saturnino C 2010, *Molecules* 15 2028.
2. Pushpangadan P, Rajasekharan S, Rathesh Kumar PK, Jawahar CRVNV, Lakshmi N and Amma LS 1988 *Ancient Sci Life* 7 13.
3. Sharma A K, Pushpangadan P, Chopra C L, Rajasekharan S and Saradmmal L 1989 *Anc Sci Life* 8 212.
4. Singh B, Gupta O K and Chandran B K 2001 *Phytomedicine*.8 283.
5. Butani, K.K., Taggi, D.K., Anand, B.S., Kapil, R.S., Pushpangadan, P. and Rajsekhran, S. (1994). A process for the preparation of a glycolipid fraction from

- Trichopus zeylanicus* possessing adaptogenic activity. Patent application No 88/Del/94.
6. Subramoniam A, Madhavachandran V, Rajasekharan S and Pushpangadan P 1997 Journal of Ethnopharmacology. 57 21.
  7. Saudagar R B, Sambath Kumar and Bachhav R S 2013 Journal of pharmacognosy and phytochemistry 5 258.
  8. Subramanian A, Evans DA, Rajasekharan S and Pushpangadan P 1998 Indian Journal of Experimental Biology. 36 385.
  9. Subramoniam, Appian, Rajasekharan, Sreedharan, Pushpangadan, Palpu, George, Varghese, Nair, Gopala Pillai Sreekandan. A process for a novel herbal medicinal composition for cancer treatment from Janakiarayalpathra root and *Trichopus zeylanicus* leaf. Indian (2010), 1N 193609 A1 20100910.
  10. Sivanandham Velavan, S. Selvarani and A. Adhithan 2009 Bangladesh. J. Pharmacol 4 88.
  11. Mohan VR, Anitha B, Perumalsami Athi and Sutra S 2008 Ethno botanical Leaflets 12 171.
  12. Tharakan Dhanasekaran M and Manyam BV 2005 Phototherapy Research. 19 669.
  13. Ramalakshmi S, Edaydulla N, Ramesh. P and Muthuchelian K 2012 Asian Pacific Jour. of Tropical Disease D68 S75.
  14. Chang CC, Yang MH, Wen HM and Chern JC 2002 J. Food Drug Anal. 10 178.
  15. Elija Khatiwora, Vaishali B. Adsul, Manik M. Kulkarni, N.R. Deshpande and R.V Kashalkar 2010 International Journal of ChemTech Research. 2 1698.
  16. Jensen A. Chlorophyll and carotenoids. In: Hallebust JA, Craigie JS. (eds). Handbook of physiochemical and biochemical methods. Cambridge: Cambridge University Press; 1978, p. 5-70.
  17. Siddhuraju P, Mohan PS and Beaker K 2009 Food chem 79 61.
  18. Dinis TCP, Madeira VMC and Almeida LM Arch Biochem Biophys 315 161.
  19. Kannan RRR, Arumugam R and Anantharaman P 2010 Asian Pacific J Trop Med 11 898.
  20. Jayaprakasha GK, Jaganmohan RL and Sakariah KK 2004 Bioorg Med Chem 19 5141.
  21. Irdafidrianny, irarahmiyani and komarruslanwirasutisna 2013 International. Jour. Of pharmacy and pharmaceutical sciences 4 189.
  22. Aquino R, Morelli S, Lauro M R, Abdo S, Saija A and Tomaino A 2001 Journal of Natural products 64 1019.
  23. Lu Y and Foo Y 2000 Food Chem 68 81.
  24. Govindarajan R, Singh DP and Rawat AKS 2007 J Pharm Biomed Anal 43 527.