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EVALUATION OF DPPH RADICAL SCAVENGING ACTIVITY OF THE LEAF, ROOT AND FRUIT EXTRACTS OF *TRICHOPUS ZEYLANICUS* FROM SOUTH INDIA

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ABSRACT

The DPPH radical scavenging activity of the petroleum ether, ethyl acetate, methanol and aqueous extracts of the leaf, root and fruit of *Trichopus zeylanicus* was invesigated. Among the leaf,root and fruit extracts of *Trichopus zeylanicus* studied, methanol extract of leaf, ethyl acetate and aqueous extracts of root and methanol and water extracts of fruit showed potent scavenging activity on 1, 1-diphenyl-2-picrylhydrazyl (DPPH) radical. The remarkable antioxidant activity exhibited by the leaf,root and fruit extracts can be attributed to the synergic effect of the active compounds present in it. Phytochemical screening of the leaf, root and fruitextracts showed the presence of phenolic compounds, flavonoids, alkaloids, steroids and tannins present in them and these compounds are reported to have antioxidant properties. The results showed that the leaf, root and fruit extracts of *Trichopus zeylanicus* can be considered as good source of natural antioxidants and can be incorporated into drug formulations.

KEY WORDS: *Trichopus zeylanicus*, DPPH radical scavenging activity, Phytochemical screening, Antioxidants, Drug formulations.

1.1 INTRODUCTION

Trichopus zeylanicus which is a perennial herb, belongs to the family Trichopodaceae popularly known as “Arogyapacha” in Malayalam literally meaning ‘the green that gives

strength". Arogyapacha is a subspecies of *Trichopus zeylanicus*. The plant has distribution in Malay Peninsula, Srilanka, Thailand and India. Only the Indian variety is proved to have medicinal qualities. It is found distributed in Southern western Ghats in the hills of Travancore "Agasthyakoodam" hilly forest of Kerala. The tribal inhabitants of this area (Kani tribes) are using plant for increasing the stamina.[1] The unripe fruits are used by Kani tribes as a highly rejuvenating tonic comparable to Ginseng. Arogyapacha was later identified as one of the celestial plants, Varahi, mentioned in one of the oldest classical texts in Ayurvedic Literature SusruthaSamhitha.[2]

The plant shows many pharmacological activities. Administration of ethanolic leaf extract to male mice stimulated their sexual behavior as evidenced by an increase in number of mounts and mating performance.[3] The alcoholic extract of seeds of *Trichopus zeylanicus* showed adaptogenic or antistress in both rats and mice. This extract also enhances swimming performance in mice.[4] Isolation of glycolipid fraction from the plant possessing adaptogenic activity.[5] Oral administration of *Trichopus zeylanicus* induces immunomodulation that protect mice from tumour cell growth.[6] *Trichopus zeylanicus* extracts have been evaluated for its anti hepatoprotective and choleretic activities in rats.[7] A glyco-peptido lipid fraction from the alcoholic extract of the herb was evaluated for anti stress activity.[8] Adaptogenic activity was showed by the glyco peptido-liquid fraction from the alcoholic extract of Trichopus zeylanicus.[9] The leaves of *Trichopus zeylanicus* is used by Kannikars for scabies and ring worm infections,[10] animal studies showed the cardio protective effect of the plant,[11] and anti-cancer activity of a herbal composition of this plant.[12]Anti-oxidant properties of crude *Trichopus zeylanicus* were established on free radicals (DPPH and ABTS), Itsiron reducing ability, lipoxygenase activity, hydrogen peroxide induced lipid peroxidation and anti-stress activities are due the presence of NADH, Polyphenols and Sulfhydryl compounds in them.[13]

There are multitudes of pharmacological potential activities documented on this plant and numerous studies have also been reported on these activities. There are yet more activities of plant to be explored. Considering the knowledge attained from literature survey, as well as by combining the tribal knowledge and the modern pharmacological works, the present work seeks to investigate the uninvestigated areas by reliable methods of DPPH Radical Scavenging assay of various extracts and also emphasizing the presence of secondary metabolites in the extracts of the plant. The results showed that the methanol extract of leaf,

ethyl acetate and aqueous extracts of root and methanol and water extracts of fruit of *Trichopus zeylanicus are* good sources of active compounds and antioxidants.

1.2 MATERIALS AND METHODS

1.2.1 Plant Material

Five hundred small herbs of Arogyapacha 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, Kerala. Voucher specimen is deposited in the specially maintained herbarium, Department of Botany, Vimala College, Thrissur, Kerala.

1.2.2 Preparation of Extracts

Fifty grams of the powered leaves, root and fruits 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.

1.2.3 Preliminary Phytochemical analysis

The sample extracts were analysed for the presence of various phytoconstituents like flavonoids, alkaloids, glycosides, steroids, phenols, saponins and tannins according to standard methods.[14,15]

1.2.4 DPPH free radical scavenging assay

The DPPH free radical is a stable free radical, which has been widely accepted as a tool for estimating free radical-scavenging activities of antioxidants.[16] The hydrogen or electron donation abilities of the compounds were measured from the bleaching of the purple-colored ethanol solution of 1, 1-diphenyl-2-picrylhydrazyl (DPPH). This spectro-photometer assay uses the stable radical DPPH as a reagent. The sample solution of material (50 µl) at four concentrations (1.0, 0.5, 0.25 and 0.125 mg/ml) was mixed with freshly prepared methanolic solution of DPPH (634 μM) and allowed to stand for 30 min at room temperature. The absorbance was then measured at 515nm using a spectrophotometer and the inhibition of free radical DPPH in percent (%) was calculated using the formula below:

The percent of inhibition of DPPH reduction (decolourization)

$$\% \text{ of inhibition} = \frac{A_0 - A_{sample}}{A_0} \times 100$$

The percent of inhibition of DPPH reduction (decolourization) where (A_0) is the absorbance of the control (blank) and (A_{sample}) is the absorbance of the test compound. The compound concentration demonstrating 50% inhibition (IC_{50}) was calculated from the plot of inhibition percentage against sample concentration. Tests were carried out in triplicate. Samples and DPPH were dissolved in methanol. L-ascorbic acid was used as positive control.[17]

1.3 RESULS AND DISCUSSION

1.3.1 Phytochemical screening

The phytochemical screening of the leaf extracts revealed that alkaloids, flavanoids, glycosides, tannin, steroids and terpenoids are present in large amount in the methanol extract. Phenolic compounds, flavonoids, tannins, terpenoids and steroids are present in chloroform extracts. The water extract contains alkaloids, flavanoids and saponins.(Table1) The phytochemical screening of the root extracts gives significant results for flavanoids, glycosides, steroids and terpenoids. Alkaloids, flavanoids and steroids are present in methanolic extracts. The water extract contains phenolic compounds, tannin and terpenoids.These phytochemicals confer antibacterial, antioxidant and anticancer activities on the root extracts.(Table2) Phytochemical screening of fruit extracts shows the presence of flavanoids, saponin, glycosides, steroids, terpenoids in methanol extract. (Table3)

Phytochemical studies revealed the presence of various secondary metabolites in the leaf, root and fruit extracts of *Trichopus zeylanicus*.The study shows the presence of tannin, terpenoids, phenols, flavanoids and saponins in significant amount in the plant extracts. The various phytochemical compounds detected are known to have beneficial importance in medicinal sciences andconfer antibacterial, antioxidant, anticancer activity on the leaf, root and fruit extractsof the plant.The results of this study support the use of this plant for human diseases and reinforce the ethnobotanical importance of plant as a potential source of bioactive substances.[18]

Kannikars, predominant hill tribes of Western Ghats and Tamil Nadu are well known about *Trichopus zeylanicus* (Arogyapacha) and its medicinal uses. Apart from the practices of allopathic drugs, these folk knowledges and folk medicines which have not been studied methodically and mostly not documented are worth assessing. The many reported pharmacological activities of the plant are due to the presence of phytoconstituents in it.[19,20,21]

1.3.2 Antioxidant activity

The antioxidant activity of theleaves, roots and fruit extracts of *Trichopus zeylanicus* extracts in solvents of varying polarity were measured in terms of hydrogen donating or radical scavenging ability, using the stable radical, DPPH. The method is based on the reduction of alcoholic DPPH· solutions in the presence of a hydrogen donating antioxidant. DPPH· solutions show a strong absorption band at 515 nm appearing as a deep violet color. The absorption vanishes and the resulting decolourization is stoichiometric with respect to degree of reduction. The remaining DPPH, measured after a certain time, corresponds inversely to the radical scavenging activity of the antioxidant.

The results of the free radical scavenging activity of the leaf, root and fruit extracts of *Trichopus zeylanicus* assessed by DPPH assay were summarized in(Tables 4,5,6). *Trichopus zeylanicus* leaf methanol extract, ethyl acetate root extract and methanol fruit extract possess potent free radical-scavenging activity. The amount of the sample needed for 50% inhibition of free radical activity is expressed by IC_{50}andlower IC_{50} value indicates higher antioxidant activity. IC_{50} values of leaf, root and fruit extracts and the authentic antioxidant L-ascorbic acid are also evaluated.

Antioxidents are resistant against the oxidative stress by scavenging freeradicals 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.[22,23]

The DPPH free radical scavenging activity of the leaf , root and fruit extracts of *Trichopus zeylanicus* are sorted in descending order: Leaf methanol extract > Leaf chloroform extract > Leaf ethyl acetate extract > Leaf water extract > Leaf petroleum ether extract. Root ethyl acetate extract > Root water extract> Root methanol extract > Root petroleum ether extract, Fruit methanol extract > Fruit water extract > Fruit chloroform extract > Fruit ethyl acetate extract > Fruit petroleum ether extract.Out of the samples tested, *Trichopus zeylanicus*leaf methanol extract showed the highest scavenging activity (% inhibition 100, 97.5, 90 and 60 at 1.0, 0.5, 0.25 and 0.125mg/ml respectively), followed by *Trichopus zeylanicus* fruit methanol extract(% inhibition 96.25,92.5,76.25 and 73.75 at 1.0, 0.5, 0.25 and 0.125mg/ml respectively) and fruit water extract (% of inhibition 93.75, 81.25, 76.25 and 72.5 at 1.0, 0.5, 0.25 and 0.125 mg/ml respectively).Root petroleum ether extract is exhibited least DPPH radical scavenging ability with % inhibition 40, 8.7, 6.25 and 2.5 at 1.0, 0.5, 0.25 and

0.125mg/ml respectively. By comparing the IC_{50} value of the leaf, root and fruit extracts of *Trichopus zeylanicus* with that of the authentic antioxidant L-ascorbic acid, it was found that the antioxidant activity of *Trichopus zeylanicus* leaf methanol extract (IC_{50}: 50 µg/ml) was quite comparable with that of L-ascorbic acid (IC_{50}: 53.15µg/ml). IC_{50} value of *Trichopus zeylanicus* fruit methanolextract (81.05µg/ml) and *Trichopus zeylanicus* fruit water extract (83.5µg/ml) are not significantly different from that of L-ascorbic acid (IC_{50}: 53.15µg/ml).

Table 1:Phytochemical analysis of leaf extracts of *Trichopus zeylanicus*

Phytoconstituent	Petroleum ether extract	Ethyl acetate extract	Chloroform extract	Methanol extract	Water extract
Alkaloids	++	++	++	+++	+++
Phenol	+	++	+++	++	++
Flavanoids	++	+++	+++	+++	+++
Tannin	-	++	+++	+++	+++
Glycosides	-	-	++	+++	+
Steroids	+++	+++	+++	+++	++
Terpenoids	+	++	+++	+++	+
Saponins	-	+	++	++	+++

+ present ++ moderately present +++ appreciable amount

Table 2:Phytochemical analysis of root extracts of *Trichopus zeylanicus*

Phytoconstituent	Petroleum ether extract	Ethyl acetate extract	Methanol extract	Water extract
Alkaloids	-	+	+++	-
Phenol	+	++	+	+++
Flavanoids	+	+++	+++	+
Tannin	-	++	++	+++
Glycosides	-	+++	+	++
Steroids	+	+++	+++	++
Terpenoids	+	+++	++	+++

+ present++ moderately present +++ appreciable amount

Table 3: Phytochemical analysis of fruit extracts of *Trichopus zeylanicus*

Phytoconstituent	Petroleum etherextrat	Ethylacetate extract	Chloroform extract	Methanl extract	Water extract
Alkaloids	+	++	++	+	++
Phenol	-	-	-	++	++
Flavanoids	++	-	++	+++	+++
Saponin	+++	+++	+++	+++	+++
Glycosides	++	++	+++	+++	+++
Steroids	-	+	+	+++	++
Terpenoids	-	+	+	+++	++

+ Present ++ moderately present +++ appreciable amount

Table 4: DPPH scavenging activity of leaf extracts of Trichopus zeylanicus

***Trichopuszeylanicus* leaf extracts**	**Concentration of sample in mg/ml**				**IC_{50} in µg/ml**
	1 mg/ml	**0.5 mg/ml**	**0.25 mg/ml**	**0.125 mg/ml**	
	Radical scavenging effect (%)				
Petroleum ether	68.75	52.5	41.25	31.25	506.9
Ethyl acetate	91.25	81.25	63.75	37.5	126.2
Chloroform	75	67.5	63.75	52.	109.36
Methanol	100	97.5	90	80	50
Water	71.25	66.2	52.5	35	298
L Ascorbic acid	97.5	96.25	95	93.75	53.19

Table 5: DPPH scavenging activity of root extracts of *Trichopus zeylanicus*

***Trichopus zeylanicus* root extracts**	**Concentration of sample in mg/ml**				**IC_{50} in µg/ml**
	1mg/ml	**0.5mg/ml**	**0.25mg/ml**	**0.125mg/ml**	
	Radical scavenging effect (%)				
Petroleum ether	40	8.7	6.25	2.5	1298
Ethyl acetate	92.5	88.75	76.25	57.5	126.8
Methanol	51.25	40	28.75	10.0	888.02
Water	92.5	98.75	55	36.27	209.69
L Ascorbic acid	97.5	96.25	95	93.75	53.19

Table 6: DPPH scavenging activity of fruitextracts of *Trichopus zeylanicus*

***Trichopus zeylanicus* fruitextracts**	**Concentration of sample in mg/ml**				**IC_{50} in µg/ml**
	1mg/ml	**0.5mg/ml**	**0.25mg/ml**	**0.125mg/ml**	
	Radical scavenging effect (%)				
Petroleum ether	62.5	43.75	31.25	0.05	721.36
Ethyl acetate	70	56.25	48.75	35	399.650
Chloroform	83.75	80	68.75	50	125
Methanol	96.25	92.5	76.25	73.75	81.05
Water	93.75	81.25	76.25	72.5	83.5
L Ascorbic acid	97.5	96.25	95	93.75	53.19

1.4 CONCLUSIONS

Phytochemical screening of plant extracts of leaf, root and fruit extracts of *Trichopus zeylanicus* shows that phytoconstiuents such as phenolic components, saponins, glycosides and steroids are present significantly high amountin methanol extract of leaf, ethyl acetate extract of root and methanol extract of fruit and these phytochemicals are reported to have antioxidant activities.[24]

Among the leaf, root and fruit extracts of *Trichopus zeylanicus* studied, the IC_{50} value of methanol extract of leaf, methanol extract of fruit, aqueous extract of fruit, ethyl acetate extract of root and chloroform extract of fruit is low.The lower IC_{50} value shows their higher free radical scavenging activity.Higher scavenging activity on DPPH free radical of the leaf

methanol extract, fruit methanol extract, fruit aqueous extract, ethyl acetate extract of root and chloroform extract of fruit can be attributed to the presence of antioxidants in them and these extracts of the plant can be incorporated into the drug formulation.

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