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GC-MS analysis of ethanolic plant extract of *Trichopus Zeylanicus* ssp. *Travancoricus* Burkill Ex K. Narayanan

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Abstract
The aim of the present study is to analyze the bioactive phytochemical constituents present in the plant of *Trichopus zeylanicus* ssp. *travancoricus* Burkill ex K. Narayanan by using GC-MS.
Methods: The dried and coarsely powdered plant materials were extracted using solvent ethanol for 5 hours by soxhlet extraction method. The presence of various phytochemical constituent was analyzed by GC-MS.
Results: Totally 22 chemical constituents were identified. Among these twenty-two compounds detected from ethanolic extract,2-methyl-3-oxy-gamma-pyrone (1.87%), 2-methyl-3-hydroxypyrone (2.77%), Pyranone (4.88%), Coumaran (12.74%), Vanillin (1.24%), Isovanillicacid (1.96%), Chinasaure (Quinic acid) (9.15%), alpha-santonin (4.98%),digiprolactone (1.46%), Ascorbic acid 2,6-dihexadecanoate (0.43%), and phenolic derivates were identified as therapeutically active components.
Conclusion: The obtained phytochemical constituents were identified as potentially active and can be used for the treatment of various diseases. Constituents supporting nutritive value were also identified.

Keywords: Arogyapacha, coumaran, kani tribe, quinic acid

Introduction
Trichopus zeylanicus ssp. *travancoricus* Burkill ex K. Narayanan is a herb belonging to the family Trichopodaceae. The plant is locally known as 'Arogyapacha' in Malayalam, 'Arogya' means health and ' Pacha' means green and hence the herb is literally called as green that gives health. Three subspecies of *Trichopus zeylanicus* are identified and among these, *Trichopus zeylanicus* ssp. *travancoricus* have reported as endemic to the Southern Western Ghats in India with a restricted distribution in Agasthyamalai Biosphere Reserve. The' Kani tribe' of Agasthyamalai has introduced various uses of this wild plant to the present medical world. They also claim that one who consumes the fruits of Arogyapacha regularly, will remains healthy, agile and disease resistant [1]. The powdered leaves of *T. zeylanicus* ssp. *travancoricus* along with stem bark of *Mangifera indica* was used to treat venereal diseases [2]. The indigenous tribal community in Agastya hills traditionally uses this plant as an instant energy booster that combat fatigue [3]. Apart from anti fatigue property, medicinal properties such as anti-oxidant, anti-inflammatory, aphrodisiac [4], hepatoprotective [5], mast cell stabilization [6], anti-hyperlipidemic, adaptogenic [7], cardioprotective [8], and anti-ulcer activity [9] were also reported to this endemic herb. Literature survey revealed that various therapeutic properties of *Trichopus zeylanicus* including anti-oxidant, anti-stress, anti-microbial, aphrodisiac, analgesic, anti-inflammatory etc., have been demonstrated using various plant extracts both in *in vitro* and *in vivo* studies [3]. This study focuses to analyze the constituents responsible for contributing various pharmacological activities and nutritive value to the plant. The results of pharmacological activities and nutritive value of this plant accentuates a point of view that helps in future research to explore its full pharmacological potential and nutritive status. The present study focused to analyze the bioactive chemical constituents present in the ethanolic extract of whole plant material of *Trichopus zeylanicus.*

Fig 1: *Trichopus zeylanicus* ssp. *travancoricus*

Materials and Methods

1. *Plant material collection and authentification*

The plant *Trichopus zeylanicus* ssp. *travancoricus*(Fig.1.) were collected from Rayirath Gardens, Pattikkad, Thrissur, Kerala, India. The specimens collected from nursery were identified with the standard literature and authenticated with valid voucher specimens. The plant materials were taxonomically identified by the Botanist, Dr. Udayan P.S, Assistant professor and Head of P G Department of Botany and Research, Sree Krishna College Guruvayoor. The specimen of *T. zeylanicus* ssp. *travancoricus* voucher number 85 was submitted in the Herbarium.

2. *Extraction*

The whole plant material was shade dried, coarsely powdered and subjected to soxhlet extraction by using solvent ethanol for 5 hours. The extract was filtered through Whatman No. 1 filter paper and concentrated. The extract obtained was then subjected to GC-MS analysis.

3. *GC -MS Analysis*

Gas chromatography Mass spectroscopy analysis of ethanolic extract was performed using Shimadzu GC-MS Model number: QP2010S equipped with Column - ELITE-5MS (30 meter length, 0.25 mm ID, and 0.25 µm thicknesses). Electron ionization system was used; details of GC programme were given in Table I. The oven temperature was programmed from 70.00^{0}C which is given in Table II. Helium gas was used as the carrier gas. Details of GC-MS programme was given in Table III. Programme specifications regarding Mass Spectra were depicted in Table IV. GCMS Software: GCMS Solutions, Libraries used: NIST 11& WILEY 8.

Table 1: GC programme (GC 2010)

GC-Parameters	Programme
Column oven temperature	70.00^{0}C
Injection temperature	260.00^{0}C
Injection mode	Split less
Sampling time	2.00 min
Flow control mode	Linear velocity
Pressure	61.5 kPa
Total flow	54.1mL/min
Column flow	1.00mL/min
Linear velocity	36.7 cm/sec
Purge flow	3.0mL/min
Split ratio	50.0

Table 2: Oven temperature programme

Rate	Temperature(^{0}c)	Hold time(min)
-	70.0	2.00
10.00	200.0	5.00
5.00	280.0	15.00

Table 3: GC-MS programme (GCMS QP2010)

GC-MS Parameters	Programme
Ion source temperature	200.00^{0}c
Interface temperature	280.00^{0}c
Solvent cut time	6.50 min
Detector gain mode	Relative
Detector gain	1.01 kV+0.00 kV
Threshold	1000

Table IV: MS table

Mass spectroscopy parameters	Programme
Start time	6-7 min
End time	51.00 min
ACQ time	Scan
Event time	0.50 sec
Scan speed	1000
Start m/z	50.00
End m/z	500.00
Sample inlet unit	GC

4. Identification of compounds

The constituents in the extract were identified by comparing their relative retention time and confirmation was done by comparing the mass spectra with database from the Library of NIST 11 and Wiley8. GC-MS Chromatogram obtained was given in figure.2.

Results and Discussion

GC-MS analysis of ethanolic extract of *Trichopus zeylanicus* were carried out and a group of 22 compounds were identified which are depicted in Table V. This includes several phenolic derivatives and other biologically and pharmacologically active compounds. The compounds identified are -2-methyl-3-oxy-gamma-pyrone (1.87%), 2-hydroxyacetylfuran (4.37%), Erythrol (3.14%), 2-ethyl crotonaldehyde (9.16%), 2-methyl-3-hydroxy pyrone (2.77%), Glycerin (6.21%), Pyranone (4.88%) 1, 2-benzenediol (3.21%), Coumaran (12.74%), 5-Hydroxymethylfurfural (8.53%), 4-vinylguaiacol (12.99%),Phenol, 2, 6-dimethoxy- (2.95%), Vanillin (1.24%), 2,6-cresotaldehyde (3.83%), 4-tert-Butoxybenzoic Acid (1.63%), Isovanillic acid (1.96%), -acetyl guaiacol (0.55%), Chinasaure (9.15%), alpha-santonin (4.98%), gamma hydroxyisoeugenol (1.96%), Digiprolactone (1.46%) - Ascorbic acid 2, 6-dihexadecanoate (0.43%). Each of these constituents is responsible for various pharmacological and biological activities. Of these 22 compounds, phenolic derivatives (4- vinylguaiacol 12.99%, phenol 2, 6 dimethoxy 2.95%, and acetyl guaiacol 0.55%) were found as the first major compounds. Phenolic derivatives are well known for their antioxidant activities. Coumaran (12.74%) were found as the second major compound [10]. Coumarans (2, 3-dihydrobenzofurans) have antitubercular, anti-HIV, antioxidant, anti stress activity [11]. Chinasaure (Quinic acid 9.15%) were found to be third major therapeutically important compound. Study suggested that the pharmacokinetic properties of Quinic acid were more preferable to be used as a potent drug candidate to combat prostate cancer [12]. Quinic acid nutritionally supports the synthesis of tryptophan and nicotamide in GI tract that in turn leads to the DNA repair enhancement and NF-kB inhibition via increased nicotinamide and tryptophan production. It also promotes insulin secretion from pancreatic beta cells [13]. Now a days pyrone and its derivatives are used for the treatment of an

Anticancer, Antibiotics, Anticoagulants, and HIVprptease etc. [14] Isovanillin posses antidiarrheal and anti motility effect on GI tract [15]. Alpha santonin are sesquiterpene lactones and are pharmacologically proved as Antipyretic, Anthelmintic, Anticancer, and Analgesic.

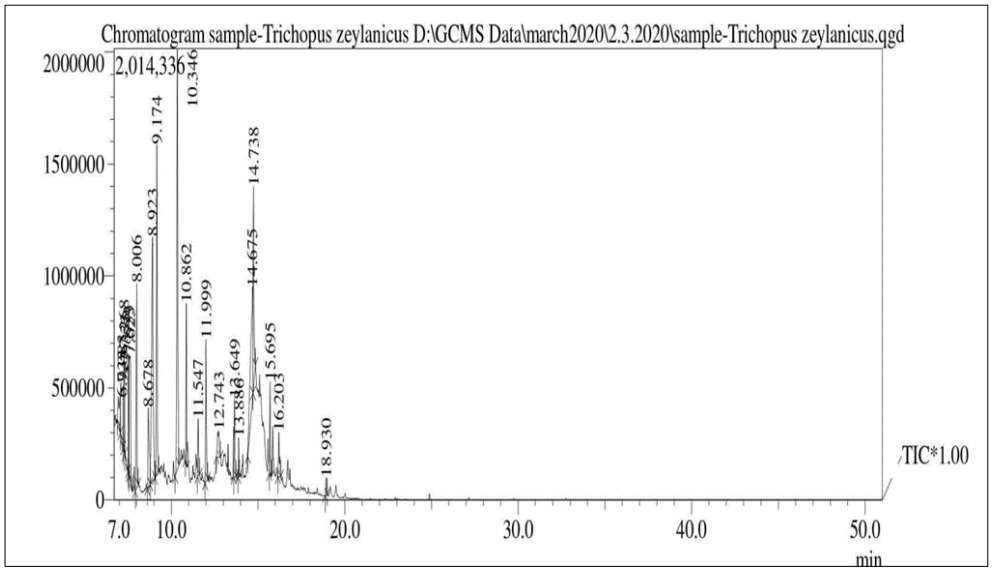

Fig 2: GC-MS chromatogram of ethanolic extract of *Trichopus zeylanicus* ssp. *travancoricus*

Table 5: GC-MS analysis of ethanolic extract of *Trichopus zeylanicus* ssp. *travancoricus*

Peak#	R. Time	Area	Area %	Height	Height%	Name	Base m/z
1	6.933	1027896	1.87	110783	0.90	2-METHYL-3-OXY-GAMMA-PYRONE	126.10
2	7.083	2407378	4.37	235712	1.91	2-HYDROXYACETYLFURAN	95.00
3	7.268	1726530	3.14	458117	3.71	ERYTHROL	57.00
4	7.333	5039753	9.16	421784	3.41	2-ETHYL CROTONALDEHYDE	61.05
5	7.533	1523675	2.77	532583	4.31	2-METHYL-3-HYDROXYPYRONE	126.10
6	7.625	3420193	6.21	558796	4.52	Glycerin	61.05
7	8.006	2686098	4.88	913469	7.39	Pyranone	144.05
8	8.678	1764178	3.21	353294	2.86	1,2-BENZENEDIOL	110.05
9	8.923	7013530	12.74	1089506	8.81	Coumaran	120.10
10	9.174	4697227	8.53	1474539	11.93	5-Hydroxymethylfurfural	97.05
11	10.346	7148565	12.99	1879333	15.20	4-VINYLGUAIACOL	150.10
12	10.862	1621732	2.95	715220	5.78	PHENOL, 2,6-DIMETHOXY-	154.10
13	11.547	681135	1.24	256415	2.07	Vanillin	152.10
14	11.999	2108978	3.83	634986	5.14	2,6-CRESOTALDEHYDE	136.10
15	12.743	895719	1.63	118388	0.96	4-tert-Butoxybenzoic Acid	57.05
16	13.649	1077092	1.96	350559	2.84	Isovanillic acid	168.10
17	13.886	300568	0.55	171595	1.39	_ACETYLGUAIACOL	151.05
18	14.675	5036008	9.15	502602	4.07	CHINASAURE	60.05
19	14.738	2742771	4.98	894566	7.24	(-)-.ALPHA.-SANTONIN	182.10
20	15.695	1080247	1.96	414287	3.35	gamma.-Hydroxyisoeugenol	137.10
21	16.203	801232	1.46	199859	1.62	DIGIPROLACTONE	111.10
22	18.930	236629	0.43	77665	0.63	l-(+)-Ascorbic acid 2,6-dihexadecanoate	73.05
		55037134	100.00	12364058	100.00		

Conclusion

In the present study, twenty two chemical constituents have been identified from the ethanolic extract of *Trichopus. Zeylanicus* ssp. *travancoricus*. The obtained bioactive compounds were identified as potentially active and can be used for the treatment of various diseases. Constituents supporting nutritive status were also identified. The presence of various phytoconstituents helps in future research to explore full pharmacological potential and nutritive value of the plant.

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Conflict of Interest

The authors have no conflict of interest.

Abbreviation Used

GC-MS: Gas Chromatography- Mass Spectroscopy; Rt: Retention time; NIST 11; National Institute Of Standard and Technology 11.

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