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Research Article

CYTOTOXIC AND ANTIMICROBIAL STUDIES ON AROGYAPACHA OR KERALA GINSENG LEAF EXTRACTS

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INTRODUCTION

Trichopus zeylanicus ssp.travancoricus Burkill.ex.Narayanan is commonly called 'Arogyapacha' or "Kerala Ginseng[1]" is a small perennial herb with rhizomatous stem belonging to the monogeneric family Trichopodaceae[2] . The unripe fruits are used by Kani tribes as a highly rejuvenating tonic comparable to Ginseng. The Kanis claim that to remain healthy, agile, young and resistant to various diseases or infections one should consume the fresh fruits of the plant. Several works were previously done in the laboratories of Tropical Botanical Garden and Research Inststute, Palode, Kerala and revealed the strong anti -fatigue properties of this plant. The pharmacological investigationshave proved antistress, stamina boosting, immunomodulatory, hepatoprotective, aphrodisiac and antioxidant properties associated with various parts of this plant. A critical survey of the Ayurvedic classics suggests that the divine drug 'Varahi' is very true to Arogyapacha. The pharmacological studies showed that all pats of the plant possess medicinal properties like inhibition of antigen induced de-granulation of sensitized peritoneal mast cells in rats ,the antioxidant and DNA protective properties, pharmacognostic activities like immuno-enhancing, antifatigue, antistress properties[3,4]. A wide scan of literature concerning *T.zeylanicus* did not showed studies on its cytotoxic and antimicrobial activities in which the present study is concentrated.

MATERIALS AND METHODS

The specimen was collected from the Guard Station, Peringamala, Thiruvananthapuram in the month of September. It was identified and authenticated at the Herbarium of Botany Dept., University College, Thiruvananthapuram , where voucher specimen(No.10003)was preserved .

Cytotoxic study

The water extract for the treatment was prepared from the fresh leaves of *Trichopus zeylanicus ssp. travancoricus*. 50g of fresh leaves was collected in the morning at 9.30am and washed well to remove dirt. Moisture was removed by blotting with sterile tissue paper. Crude extract was prepared by grinding the leaves using a mortar and pestle. Then, it was filtered using a double layered cheese cloth. Different percentages of the extract was prepared, i.e, 1%, 2%, and 5%. The prepared extracts were taken in small labeled glass bottles. Sprouted onion bulbs placed in sunlight for 30 minutes were placed in these bottles at 10 a.m having 1%, 2%and 5% solutions for 2hr, 4hr, 6hr and 24hr. After this treatment the root tips were fixed in fixative fluid, 1 acetic acid: 3 ethyl alcohol. Control was kept, i.e. treating the root tips in distilled water for the same duration. The root tips were hydrolyzed in 1N hydrochloric acid for 1 min at 50-60° C, then washed well and squash preparations were made in 2% acetocarmine. Scoring of cells showed various cellular aberrations both clastogenic and nonclastogenic. Estimation of percentage of aberrations and mitotic indices were calculated using the standard parameters[5].

Antimicrobial studies

500g of cleaned leaves were shade dried for 45 days. These were finely powdered. 30g of the dried powder were used for continuous distillation process using soxhlet apparatus. The solvents used in partial distillation were Hexane, Chloroform, Methanol. The extracts were made by series distillation i.e, on the first day, continuous 8hr by hexane, followed by 8hr by chloroform and 8hr by methanol during third day. These three extracts were kept separately ,labeled and let the solvent to be evaporated, so that crude extract will remains. Filter paper disc diffusion method was used for the *invitro* evaluation of antimicrobial activity[6]. 20ml of

sterilized medium was taken in each Petri dish. After the medium had hardened 2ml of 24hr broth culture of sub-cultured microbes was distributed evenly over the surface of the petriplate medium and mixed thoroughly by rotatory motion of the plate medium and allowed to set. The sterilized Whatman No. 42 disc(4mm diameter)were thoroughly moistened with extracts distilled using different solvents through distillation(Hexane, Methanol, Chloroform). The standard discs of Potassium penicillin G(1000ppm) and Streptomycin sulphate(1000ppm) were placed as standard for antibacterial activity and Greseofulvin (1000ppm) were placed as standard for antifungal activity. Discs moistened with hexane, Chloroform and Methanol were also placed separately as control for the three different solvent distillation.

The Petri plates were inverted and placed in an incubator for 36hr at 37EC in case of bacteria and 72hr at 27EC in case of fungi to obtain perfect growth. After incubation the relative susceptibility of the organism to the extract was demonstrate by a clear zone of inhibition around the disc. The zone of inhibition was measured and their minimum inhibitory concentrations in mg/ml were determined. All the tests were conducted in three sets for each microbes. The average results for antibacterial and antifungal activity were recorded. Pure samples of eight bacterial strange were obtain from Dept. of Microbiology , Medical college, Thiruvananthapuram and pure samples of eight fungal strains were obtain from Dept. of Pathology, Agricultural University Vellayani, Thiruvananthapuram.

Estimation of total phenols and phenolic components

Total phenol contents of the leaves were estimated by the method of Mayr *et al* [8]. The total phenol/g tissue was calculated from the standard graph.

The phenolic components of extracts were separated using RP-HPLC following the method of Beta *et al* [9]. standard phenolic acid sample such as Coumarate, Feroleate, Chlorogenate, Caffeate were injected in to the column separately. Comparing with the retention time of the standard phenolic acids in the sample was identified. Height of the peaks was taken for quantification.

RESULTS AND DISCUSSION

Cytotoxic studies

The data of mitotic indices with 1%, 2% and 5% water extract treatments for different durations of time such as 2hr, 4hr, 6hr and 24hr in onion root meristem was consolidated in Table 1. A control was also maintained to compare the range of mitotic indices with the treatment. The data clearly reveals that the different concentrations of the extracts. In 1% treatment duration significantly decreases the mitotic index from a range of 10.5 for 2hr, 3.43 for 4hr, 1.30 for 6hr and 0.27 for 24hr. In the case of 2% it was 8.12 for 2hr, 2.45 for 4hr, 0.88 for 6hr and 0.14 for 24hr treatment whereas for 5% treatment the mitotic index was 4.35 for 2hr, 1.98 for 4hr, 0.54 for 6hr and 0 for 24hr. after 24hr the root tips were completely blackened and damaged. The frequency of cytological abnormalities varied from 8.92(2% 2hr) and 44.85(5%24hr)(Table 1)

There was a positive correlation observed between the percentage of abnormalities with the increase in concentration of extract and time factor. It was noticed that higher concentrations induce disintegration of nuclei and lysis of cell wall and cell membrane. A wide range of chromosome abnormalities were seen in onion root tip cells after treatment with various concentrations of leaf extract. They induce both clastogenic and non-clastogenic abnormalities. The major clastogenic abnormalities observed include nuclear lesions, chromosome stickiness, multi polarity, chromosomal laggards, diagonal metaphase and anaphase, ball metaphase, bi nucleate and tri nucleate cells, misorientation of chromosomes, non-synchronized movement of chromosomes and disturbed metaphase and anaphase. All these effects may be due to the direct or indirect action of the bioactive principles fond in the leaf extract on DNA associated proteins and mitotic spindle apparatus[5]. Earlier experimentation proved that onion root tip assay has an excellent correlation with mammalian systems[8]. Thus from the results of this study it is suggested that the crude extract possess potential cytotoxic as well as cytostatic activities. This characteristics can be exploited as an ant icancerous agent after a detailed analysis of the phytochemical components and secondary metabolites found in the leaves[9,10,11].

Antibacterial studies

The present study indicates that the extracts from the leaves showed positive reactions against microbes up to certain extent. The results of the present study was depicted in Table: 2 &3.

Antibacterial study

Of the eight strains of bacteria studied most pronounced effect was shown by methanol extract. The hexane extract showed moderate

effects against the +ve strains viz, *Staphylococcus aureus* and *Bacillus subtilis*. Under the –ve strains *Salmonella typhi* and *Streptococcus pneumoniea* cultures were also moderately inhibited by hexane extract. The chloroform extract did not showed notable inhibitory effects against the various cultures (Table:2).

Antifungal study

Under the antifungal studies also most effective inhibitions were shown by methanol extracts. The hexane extracts showed moderate inhibition against *Alternaria sps., Fusarium solani* and *Trichophyton mentagrophytes*. Chloroform extracts proved inhibitory effects against *Alternaria sps*.and *Helminthosporium sps.*(Table:3)

Biochemical studies

The amount of total protein content estimated from the mature leaf tissue of *T.zeylanicus* by making slight modifications in the Lowry's methods 14.12µg/g.

The amount of total carbohydrate present in 1g of mature leaf tissue of *T.zeylanicus* is 11.3µg/g.

The amount of total sugar content in 1g of mature leaf tissue of *T.zeylanicus* is 3.2µg/g.

Total amino acid content by the method of Moore and Stain in 1g mature leaf tissue is 6.8µg/g.

Among the different antinutritional analysis total phenolic content is estimated, is 1.47µg/g. The total phenolic content was fractionated in RP-HPLC to know the profile of phenolic acids in mature leaf tissue. The major phenolic acids include; Coumarate(1.1µg/g), Chlorogenate(0.45µg/g), Feroleate(0.2 µg/g) and Caffeate(0.498µg/g)

These phenolic acids are effective anti-oxidants because they scavenge reactive oxygen species, trap nitrate and prevent formation of mutagenic N.nitroso compounds and also have metal chelating properties[12]. However many plants with phenolic content have been reported to possess potential medicinal properties including anti-oxidant activities[13]. The previous studies already revealed the anti-oxidant and DNA protecting properties of *Trichopus zeylanicus*[14]. They have already reported the polyphenols, sulfhydral compounds, flavanoids etc.are present in this plant. The polyphenols have anti-oxidant and iron chelating properties and can combat oxidative stress. Besides polyphenols, the sulfhydral compounds also show similar activities. Aluwalia *et al*[15] and Latha *et al* [16] reported antibacterial and antifungal properties of these bioactive principles. Susan *et al*[14] separated five similar bioactive compounds from the whole plant of *Trichopus zeylanicus*. The presence of phenolic compounds was established in the present study also. Hence it is believed that these compounds may be responsible for the cytotoxic, cytostatic and antimicrobial activities of this plant.A thorough study is required to establish more facts

Table 1: Cytotoxic studies

Time In Hours	Percentage of treatment	Percentage of abnormality	Mitotic index	control mitotic index
2	1%	8.92	6.9	17.2
	2%	9.9	8.2	
	5%	10.2	4.3	
4	1%	10.5	3.4	16.4
	2%	13.5	2.4	
	5%	15.8	1.9	
6	1%	17.1	1.3	16.1
	2%	22.3	0.8	
	5%	23.89	0.6	
24	1%	38.4	0.2	11.3
	2%	39.8	0.1	
	5%	44.85	0.0	

Table 2: Invitro antibacterial activity of *trichopus zeylanicus* leaf

S.No	Bacterial strain	Name of solvent & conc. of extract (diameter of inhibition in mm)									Control solvent	Standard
		Hexane			Chloroform			Methanol				Potassium penicillin + 1µg/ml
		1µg/ml	2µg/ml	3µg/ml	1µg/ml	2µg/ml	3µg/ml	1µg/ml	2µg/ml	3µg/ml		
1	*Staphylococcus aureus*	8	9	14	3	4	11	3	9	16	-	22
2	*Bacillus subtilitis*	7	8	11	2	6	10	4	11	17	-	20
3	*Salmonella typhii*	6	7	10	4	6	10	3	16	19	-	Streptomycin 1µg/ml 21
4	*Shigella flexnori*	3	4	6	2	5	6	4	10	11	-	18
5	*E. coli*	3	4	6	2	4	8	4	12	16	-	19
6	*Klebsiella pneumoniae*	4	6	9	2	5	7	3	11	13	-	16
7	*Streptococcus pneumoniae*	6	8	12	4	5	7	3	9	12	-	15
8	*Clostridium tetani*	3	5	6	3	5	6	2	8	10	-	12

Table 3: Invitro antifungal activity of *trichopus zeylanicus* leaf

S.No	Fungal strain	Name of solvent & conc. of extract (diameter of inhibition in mm)									Control solvent	Standard
		Hexane			Chloroform			Methanol				greseofulvin 1µg/ml
		1µg/ml	2µg/ml	3µg/ml	1µg/ml	2µg/ml	3µg/ml	1µg/ml	2 µg/ml	3 µg/ml		
1	*Aspergillus fumigatus*	2	4	5	3	4	4	3	6	9	-	10
2	*Aspergillus niger*	2	3	6	3	3	5	4	5	9	-	12
3	*Penicillium spp.*	3	4	5	3	4	5	4	5. 6	7	-	11
4	*Alternaria spp.*	5	8	9	2	5	8	6	9	14	-	23
5	*Candida albicans*	2	3	5	2	3	5	4	7	8	-	09
6	*Fusarium solanii*	4	7	9	3	6	8	2	5	11	-	20
7	*Trichophyton mentagrophytes*	3	5	7	2	5	6	2	7. 6	14	-	18
8	*Helminthosporium spp.*	3	5	6	3	6	10	3. 2	5	11	-	16

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