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STUDIES ON THE ADAPTOGENIC AND ANTIBACTERIAL PROPERTIES OF POLYSCIAS FRUCTICOSA (L) HARMS

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INTRODUCTION:

In the present study the adaptogenic activities of the saponin fractions of the leaves and roots of *Polyscias fruticosa* (L) Harms (Araliaceae) were studied in comparision wit white panax ginseng root saponins. The antibacterial activity of the polyacetylenic compound in leaves were also studied. The adaptogenic activity studies showed that polyscias fruticosa leaf and root saponins have effective abtistress activity as compared with the white Panax ginseng root saponins. The antibacterial study revealed that the polyacetylenic fraction present in polyscias fruticosa leaf and root saponins have effective abtistress activity as compared with the white panax ginseng root saponins. The antibacterial study revealed that the polyacetylenic fraction present in polyscias fruticosa leaves has got better antibacterial property compared to the saponin fraction.

Mainly adaptogenic drugs are used to enhance immunity against diseases, reduce mental stress ad strain, impart a euphoric effect, retard ageing processes etc., sometimes these drugs are used as a tonic to gain non-specific resistance against various ailments[1,2]. Members of the araliaceae family contain triterpenoid saponins[3,4] Many chemical investigations on the triterpenoid saponins of the members of the members of the family revealed that the saponin content in these plants play an important role in pharmacological activities like stimulation of CNS. Reduction of fatigue ad enhancement of non-specific resistance[5,6.] Literature survey sowed that five polyactylenic alcohols are present in the root of ployscias frusticosa[7,8.] We have isolated these polyacetylenic fraction and screened its antimicrobial activity. The n-butanol fraction (mainly containing triterpenoid type of saponins) was screened for adaptogenic activity by set oexperiments like forced locometer activity, beavioural despair test, righting reflux test, swimming performance test, hypoxia test, hypethermia test immobilization stress ulceration, anabolic effect and immunostimulant activity. These activities of polyscias fruticosa saponins were compared with those of the root saponins of white panax gingseng.

EXPERIMENTAL

PLANT MATERIAL

Polyscias fruticosa was collected in the month of November- December India and authenticated by Dr. Arumugham at Dept. of Horticulture, Tamil Nadu agricultural university, Coimbatore, voucher specimens were deposited at the herbarium of the dept of pharmacognosy, college of pharmacy, SRIMPS, Coimbatore

EXTRACTION OF THE SAPONIN FRACTION [9,10]

500gm of the polyscias fruticosa leaves were powdered coarsely and soxhlet extracted for 24 hours wit 70% ethanol. The extract obtained (70 gram) was concentrated by vaccum distillation. A part of this extract was diluted with water and again extracted with chloroform to remove lipid materials. The aqueous fraction left behind was extracted further with ethyl acetate and then with n-butanol layer was separated ad evaporated to dryness (14.46 grams). The extract was found to contain saponins this extract was designated as NBHS (n-butanol extract containing saponins). In a similar manner the saponins of polyscias fruticosa roots were also extracted (yield 25.45 grams). Thin layer chromatography of both NBES leaf and NBES root over silica gel sowed 6 spots for leaf extract and 8 spots for toot n-butanol extract using solvent system n-butanol-Acetic acid – water (40:10:10). Detection UV -254 nm, NBES HR values range – 30-93; NBES root ^{leaf} HR $^{f}_{f}$ values range 10-77.

Extraction of Polacetylenic components [11,12]

1 kg of the fresh leaves of polyscias fruticosa was crushed and powdered coarsely. The crushed leaves were macerated 2 weeks with water. The whole macerate after filtration was concentrated at 50^{o}C under vacuum, to ontain an extract of semi solid consistency. The above extract was further extracted with absolute alcohol for 1 hour. This was re-extracted with 50ml of diethyl ether and concentrated under vacuum (yield 45 gm). This extract was found to contain mainly polyacetylenes and hence designated as EPA extracts (ether extract containing polyacetylenes).

The thin layer chromatographic analysis of the EPA fraction using solvent system petroleum either – acetone-Ethyl acetate (40:1:1) 0.4% Isatin in Con: H_2SO_4 (detecting agent). Showed 9 spots (Rf value range 0.27 – 0.95)

Acute toxicity studies [13]

Swiss albino mice (20-25 gms) were used for this study. These extracts EPA (Vehicle: PEG 200; 2%) NBES $_{(leaf)}$ and NBES$_{(root)}$ (Vehicle 0.5% CMC) were administered orally in doses of 0.25, 0.5, 1.0,1.5,2.0,2.5 gms.kg. All the animals indifferent drug groups were observed at regular intervals of one hour for a period of 24 hours. Toxic symptoms were observed for EPA at a dose of 1.50 gm/kg. But no toxic symptoms observed for NBES $_{(leaf)}$ and NBES $_{(root)}$ upto a dose of 2gms per Kg body weight.

Antibacterial screening studies for EPA and NBES extracts[14]

The study was carried out at the microbiology laboratory, SRIPMS, Coimbatore by the one of inhibition methods. Both NBES & EPA fractions were taken at concentrations of 10 mg/ml and 50mg/ml using dimethyl formamide as solvent vehicle. EPA and NBES extracts were tested against staphylococcus aureus, Bacillus subtilis and E.Coli. The results were compared with the reference standa5rd amoxicillin 10 mcg/ml

Adaptogenic activity studies on the saponin fraction [15,16,17,18,19]

Dose schedules

NBES $_{(leaf)}$ and NBES $_{(root)}$ 250 mg/kg; 500 mg/kg

Solvent Vehicle: 0.5% CMC

With panax ginseng root saponins: 250 mg/kg

(i) Forced Iocomotor activity

This activity was screened by observing the muscle grip strength of mice in a rota rod apparatus. Swiss albino mice of either sex (25-30gms) ere used for the screening. They were divided into four groups of sex each. The rota rod speed was adjusted to 36 rpm and the fall off time for each animal was noted down. All the animals groups were given different drug doses as per the schedule. The animals were kept undisturbed for 1 hour ad diazepam at a dose of 5mg/kg was given to all the animal groups. After 30 minutes from the time of injection the rota rod was turned on and the fall off time of animals before and after diazepam treatment was noted. The results were tabulated in table-2

(ii) Righting reflux test

Swiss albino mice of either sex weighing between 25-30 gm were used for this study. Drug extracts were given as per the schedule. Phenobarbitone sodium at the dose level of 60mg/kg i.p was given to all the group of animals. After 30 minutes from t time of injection noted the time of onset of action as t animals lost their righting reflux. The time of recovery from sleep was noted; i.e. to retain its normal posture which gives the duration of action. The results are tabulated in table-3

(iii) Swimming performance test Behavioural despair test

wistar albino rats of either sex (100-200gms) were given the schedule of drugs for five days. Animals were divided into five rats per group. On the fifth day one hour after the drug treatment, rats in all the groups were made to swim in water bath (20x8x18cm) fitted with a rotating wheel with paddles which rotate when the rats balanced and hold themselves on the wheel, rotation of the wheel was counted by a counter attached to the wheel, the rats were made to swim till they exhausted and stopped swimming. Swimming scores were recorded for individual rats are tabulated in table 4

(iv) Swim stress Induced Immobility

Swiss albino mice of either sex weighing (20-30 gms) were used for this study mice were divided into six groups each containing 6 animals drug extracts ere given as per the schedule. The animals were left undisturbed for one hour. The animals were left undisturbed for one hour. The mice were made to swim in a glass jar (25x25x12cm) containing fresh water at room temperature (25^{o}+1.0^{o}C) the water level was maintained constant at 15 cm throughout the experiment the mice were initially allowed to swim for 10 minutes and thereafter, the total period of immobility was characterized by complete cessation of swimming; with the head just floating above the water level during the subsequent 5 minutes. Tis immobility period after initial attempts to escape was postulated to represent behavioral despair, the duration of immobility of mice due to swim stress was noted and tabulates in table-5

(v) Hypoxia test:

This study was performed as per the method of collard et al21. Swiss albino mice were divided into 6 animals per group. They were given drug extract according to the dosage schedule fixed. The animals were kept in an empty glass jar of 350 ml capacity fitted with a glass stopper and made ait tight till death due to hypoxia occurred one

animal group was kept as the control and had given 0.5% CMC (solvent vehicle). The time taken for survival was recorded for the control and the drug treated groups Table -6

(iv) Hypothermia test

Swiss albino mice were selected for this study. They were divided into different drug groups of five each. Normal rectal temperature was taken using a digital telethermometer. After recording the normal temperature the animals were given NBES as per the schedule. All the mice were allowed to swim continuously for5 hours in 6" deep water kept in a cylindrical glass jar. Rectal temperature were observed again at the end of the swimming session and 30 minutes later to find out the recover from hypothermia. All the treated groups were compared with the control Tale -7.

(vii) Anabolic effect

NEBS 250 mg/kg were fed orally to wistar albino rats (150-200 gms) for a period of four weeks. The body weight of each animal was recorded daily using a digital balance. Normal diet was given to all the animal groups. The results were compared with the control group.

(viii) Immobilization stress ulceration[1]

Stress was induced by tying the limbs of the albino rats to a wooden board for a period of five hours. Drug extracts were given according to the dos schedule before inducing the stress. After five ours all the animals were killed using anesthetic ether. The abdominal cavity was opened and the stomach was excised the stomach was opened along the greater curvature, cleaned in normal saline and examined for the degree of ulceration. The ulceraogenic indices were determined cording to the following pattern.

Type of ulcer	**Score**
Minute sporadic punctuate lesions	0.5
Several small lesions	1.0
One lesion of large extension or multiple moderate sized lesions	2.0
Several large lesions	3.0

Mean ulcer score for each animal group was calculated and compared with the solvent vehicle control. Results were tabulated in table -8

(B) Anti inflammatory activity[21]

The anti inflammatory activity of the saponin fraction of leaf and root of polyscias fruticosa was studied using egg-white induced paw oedema method. The activity was compared with the standard drug phenyl butazone 100 mg/kg orally. Table -9

(C) Immuno stimulat activity [23]

(i) carbon clearance test (Determination of phagocytic index)

Swiss albino mice of either sex weighing between 25-30 gm were used for these studies. NBES $_{(leaf)}$ and NBES $_{(root)}$ were

given a dose level of250 mg/kg orally. This scheduled doses were administered to all the groups except the control group orally to 15 says prior to the injection of carbon particles. On the 16 day the animals were injected with0.1ml of carbon suspension (Pelikan tinschea Ink Germany) intravenous through the tail vein. Blood samples were collected from the retro orbital plexuses immediately at 3,5,9,12 and 15 minutes after the injection of carbon suspension. Blood samples of 25mcl were collected from all the animals and were paralysed wit 2 ml of 0.1 % acetic acid and then measured for absorbance spectrophotometrially at wave length of 675 nm. The graph of absorbance against time was plotted for each animal in the respective group . the phagocytic index is the slope of the time concentration curve and calculated for each group and expressed as mean ± standard deviation Table -10

(D) Milk –induced leukocytosis

Leucocytosis was induced by o.1 ml milk (injected subcutaneously) into swiss albino mice (20-25 gms) Blood samples were collected from the tail vein and leukocyte count was determined. Different doses of NBES (50,100,250 mg/kg) were administered orally along with the milk injection for a period of three days and the dose of NBES required for 50% reduction in leukocytosis (PD_{50}) was determined. White panax gingseng root saponin as used as a reference standard. The normal leukocyte count was observed as 4400-4500 for mouse.

Results

Acute toxity studies revealed the safety on $NBES_{(Leaf)}$ and $NBES_{(root)}$ up to 2.5 gms/kg orally. It was observed that EPA (ether extract containing polyacetylene) did not produce any toxic symptoms up to 1 gm /kg orally in a single dose.

The antibacterial effect against gram –ve and gram-ve bacterial indicated that EPA has go effective antibacterial activity compared to NBES.

(A) Adaptogenic activity screening studies:

(i) Forced locomotor activity

$NBES_{(root)}$ showed effective decrease in the fall off time as compared to the control. $NBES_{(root)}$ sowed 33.6% reduction in fall off time; while white panax ginseng root saponins showed 44.5% reduction in fall off time.

(ii) Righting reflux test

The time of recover from sleep (duration of action) for the $NBES_{(leaf)}$ And $NEBS_{(root)}$ were observed as dose dependent. NBES (root) 500 mg/kg oral dose reduced the duration of action by 48.4%. With panax ginseng root extract (250 mg/kg oral dose) reduced the time of recovery from sleep by 49.95% compared to the control group.

(iii) Swimming performance test

$NBES_{(root)}$ 500 mg/kg oral dose sowed a swimming score 77.5 ± 1.22 as compared to the control (55.42 ± 2.76). NES (leaf 500 mg/kg oral dose showed a swimming score of 75.8 ± 11.72 (control 60.3 ± 0.516

(iv) Swim stress induced immobility

$NBES_{(leaf)}$ (500 mg/kg) showed increased duration of immobility (248.5 ± 1.643 seconds) as compared to the control group (181 ± 2.97 seconds).

(v) Hypoxia test

In this study $NBES_{(root)}$ (500 mg/kg) showed effective survival time (46.0' + 1.36) as compared with the control (36.4' ± 0.30') white panax ginseng root saponin extract (250 mg/kg) showed a survival time of (44.5' ± 0.83')

(vi) Hypothermia test

$NBES_{(leaf)}$ $NBES_{(root)}$ (500 mg/kg) oral dose did not show any marked time or recovery for the rectal temperature after hypothermia induction as compared to the control group.

(vii) Anabolic effect

The body weight of $NBES_{(leaf)}$ $NBES_{(root)}$ (500 mg/kg p.o) treated animals were increased effectively as compared with the control group of animals. $NBES_{(root)}$ Treated group sowed 20% increase in body weight as compared to the control group. While $NBES_{(leaf)}$ treated group sowed 11.97% increase in body weight as compared to the control.

(viii) Immobilization stress ulceration

Ulcerogenic index for $NBES_{(leaf)}$ (500 mg/kg) was 7.045 ± 0.0288 while $NBES_{(root)}$ (500 mg/kg) showed 4.815 ± 0.0264 as the ulcerogenic index when compared to the control (13.545 ± 0.042)

(B) Anti-inflammatory activity studies

$NBES_{(leaf)}$ (500 mg/kg) showed 54.14% reduction in egg white-induced paw oedema, compared to the 71.95% reduction by phenyl butazone (100mg/kg oral dos). The reduction in oedema produced by NBES(root) 500 /kg p.o was found to be significant (67.31% reduction in oedema).

(C) Immunostimulant activity (Carbon clearance test)

It was observed that NBES (root) caused a mean phagocytic index (P.1) of 0.0426 as compared to white panax gingseng P.1 (0.0337) and NBES (leaf) P.1 (0.0266). The control group had he P.1 of 0.0152.

(D)Milk induced leukocytosis:

The dose of NBES (leaf) required to reduce the leukocyte count to 50% was found to be 234 mg/kg p.o for three days along with the milk injection. But the dose of NBES(root) required to reduce the leukcoytosis to 50% was found to be 186 mg/kg p.o for three days. White panax ginseng root gave value of 99 mg/kg for reducing leukocytosis to 50%.

Discussion

The concept of non specifically increased resistance (SNIR) was first advanced by Lazarev[23] who termed the active substances causing SNIR as adaptogens. The adaptogenic activity studies revealed that Polyscias fruticosa leaf and root saponins possess effective anti-stress activity as compared with the white panax ginseng root saponins. In the present study the drug treated animals showed better results ad compared to the control in all physical and chemical stress induced experiments (forced locomotoractivity, behavioural despair test, Righting reflux test, swimming performance test etc.,) polyscias saponins showed marked reduction in stress-induced ulceration. It was also observed that the anti inflammatory action of polyscias saponins were effective in acute models of inflammations. The immunostimulant activity studies revealed that the polyscias saponins can be effectively used as a good substitute for white panax ginseng. All these observations

clearly indicate that polysicas saponins increased the adaptability of rats and mice to stress by increasing the resistance of the animal to different stress situation nonspecifically. The adaptogenic activity of the saponins of polyscias frutocosa leaves and roots may also be due to modulation of endocrine or autocoid system to counteract stress conditions or induce immune system to produce antibodies, opsonins or interferons like substances for developing better defence against diseases.

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TABLE -1 ANTIMICROBIAL ACTIVITY STUDIES

Group Micro Organism	**Zone of inhibition in mm**				
	EPA 10mg/ml	EPA 50mg/ml	NBES 10mg/ml	NBES 50mg/ml	Amoxycillin 100mcg/ml
Stapylococcus Aureus	7.75± 0.288	9.8 ± 0.836	5.5 ± 0.5	8.16 ± 0.286	14.66 ± 0.577
Bacillus subtilus	9.23 ± 0.251	11.0 ± 0.547	7.3 ± 0.577	7.6 ± 0.578	15.7 ± 0.58
E.Coli	9.1 ± 1.01	13.0 ± 0.707	7.6 ± 0.578	8.16 ± 0.763	17.6 ± 0.577

Vehicle : DMF (Dimethyl formamide)
N=3 trials per organism

TABLE -2 FOR LOCOMOTOR ACTIVITY SCREENING STUDIES

	Fall off time in seconds			
Groups	**Dose mg/kg (oral)**	**Before Diazepam admin**	**After Diazepam admin**	**% decrease in fall off time**
$NBES_{(leaf)}$	250	111.33±2.16	61.83±7.2	12.4%
$NBES_{(root)}$	250	104.0±1.516	70.8 ±2.40	28.72%
$NBES_{(leaf)}$	250	109.5±1.516	66.3±2.36	20.5%
$NBES_{(root)}$	500	110.5±1.224	76.57±2.99	33.62%
White panax ginseng root saponins	250	109.3±3.72	79.5±3.72	44.5%
Solvent Vehicle				
Control 0.5% CMC	-	51.8 ± 3.125	51.8± 3.125	-

All the animals groups received diazepam 5 mg/kg

TABLE -3 RIGHTING REFLUX TEST

Drug Groups	Dose (oral)	Onset of action (mts)	Duration of action (mts)	Percentage decrease in duration with control
$NBES_{(leaf)}$	250	14.7+0.48	36.6+2.37	25.45%
$NBES_{(root)}$	250	13.16+1.47	32.0+1.78	38.42%
$NBES_{(leaf)}$	250	14.9+0.37	26.8+1.55	45.4%
$NBES_{(root)}$	500	15.0+0.57	25.3+0.74	48.47%
White panax ginseng root saponins	250	13.7+1.25	24.6+1.96	49.9%
Solvent Vehicle	0.5%CMC	14.2+0.48	49.1+2.53	

N=6 All the drug groups were given phenbarbitone sodium 60mg/kg i.p Value <0.05* student –t-test.

TABLE -3 RIGHTING REFLUX TEST

Drug Groups	Dose (oral)	Onset of action (mts)	Duration of action (mts)	Percentage decrease in duration with control
$NBES_{(leaf)}$	250	14.7+0.48	36.6+2.37	25.45%
$NBES_{(root)}$	250	13.16+1.47	32.0+1.78	38.42%
$NBES_{(leaf)}$	500	14.9+0.37	26.8+1.55	45.4%
$NBES_{(root)}$	500	15.0+0.57	25.3+0.74	48.47%
White panax ginseng root saponins	500	13.7+1.25	24.6+1.96	49.9%
Solvent Vehicle	0.5%CMC	14.2+0.48	49.1+2.53	

N=6 All the drug groups were given phenbarbitone sodium 60mg/kg i.p Value <0.05* student –t-test.

TABLE -4 SWIMMING PERFORMANCE TEST BEHAVIOURAL DESPAIR TEST

Drug Groups	Dose mg/kg	Swimming time (mts)	Swimming score Mean +/-SD
$NBES_{(leaf)}$	500	6.24”	75.8 ±11.72 (control score 60.3 +0.516)
$NBES_{(root)}$	500	5.26”	77.5±1.22 (Control score 55.42 +2.76)
White panax ginseng root saponins	500	5.15”	78.16 ± 1.329 (control score 59.8 +1.46)
Solvent Vehicle0.5% CMC	---	6.5”	62.5 ± 2.07

N=5

TABLE -5 SWIM STRESS INDUCED IMMOBILITYTEST

Drug Groups	Dose mg/kg (oral)	Duration of Immobility in seconds
$NBES_{(leaf)}$	250	199.6 ± 4.131
$NBES_{(root)}$	500	230.5 ± 2.18
$NBES_{(leaf)}$	250	216 ± 3.741
$NBES_{(root)}$	500	248.5 ± 1.643*
White panax ginseng root saponins	250	256.6 ± 5.85*
Solvent Vehicle	0.5%CMC 1ml/Kg	181.8 ± 2.97

N=6 Student test P Value<0.01** Value <0.05*

TABLE – 6 HYPOXIA TEST

Drug Groups	Dose mg/kg (oral)	Duration of Immobility in seconds
Control 0.5% vehicle	1ml/100gm	36.4 ±0.30
$NBES_{(leaf)}$	500 mg/kg	39.23 mg/kg 2.30
$NBES_{(root)}$	500 mg/kg	46.0 mg/kg 1.34
White panax ginseng	250 mg/kg	44.5 mg/kg 0.83

N=6

TABLE -7 HYPOTHERMIA TEST

Drug Groups	Dose mg/kg p.o	Normal Rectal temperature (*c)	Rectal temperature after 5 hours swimming session	Rectal temperature after 30 minutes of swimming session
$NBES_{(leaf)}$	500	34.4 ± 0.418	31.6 ± 1.36	32.3 ±1.035
$NBES_{(root)}$	500	34.4 ± 0.57	30.0 ± 0.894	32.6 ± 0.686
Vehicle Control 0.5% CMC		34.41± 0.57	31.4 ± 1.14	31.0 ± 0.707

N=6

TABLE -8 IMMOBILIZATION STRESS ULCERATION

Groups	Dose mg/kg	Mean Ulcer score per group	Ulcer Incedence	Ulcer index
$NBES_{(leaf)}$	250	2.6± 0.418	52%	8.328 ±0.0221
$NBES_{(root)}$	250	2.8 ±0.2738	56%	8.96 ±0.0238
$NBES_{(leaf)}$	500	2.2 ±0.274	44%	7.045 ±00288
$NBES_{(root)}$	500	1.5*± 0.353	30%	4.815* ±0.0264
Solvent Vehicle control 1% CMC		5.0 ±0.353	100%	13.545 ±0.042

N= 5 Route of administration: Oral
Student – test P value <0.05*

TABLE -9 EFFECT OF NBES ON EGG WHITE INDUCED PAW OEDEMA

Dose groups	Dose (mg/kg) p.o	Mean paw volume	Inhibition of oedema %
Control vehicle	0.5% CMC*	0.41 ± 0.03	---
$NBES_{(leaf)}$	250 mg/kg	0.318 ± 0.0045	21.95
$NBES_{(leaf)}$	500 mg/kg	0.188 ± 0.0044	54.14
$NBES_{(root)}$	250 mg/kg	0.295 ± 0.031	28.05
$NBES_{(root)}$	500 mg/kg	0.134 ± 0.30	67.31*
Phenyl butazone	100 mg/kg	0.115 ± 0.0031	71.95**

N= 5 Student – test p value <0.05
** P Value <0.01

TABLE – 10 CARBON CLEARANCE TEST

Absorbance obtained after the administration of carbon suspension								
Drug groups	**Dose**	**3mts**	**6mt**	**9mt**	**12mt**	**15mt**	**Fall in absorb ance**	**Phagocy tic index**
$NBES_{(leaf)}$	250	0.736 ± 0.00208	0.621± 0.0055	0.439 ±0.002	0.426± 0.0015	0.387 ±0.001	0.349	0.0266 ±0.00213
$NBES_{(root)}$	250	0.620 +0.001	0.478 ± 0.00152	0.327 ± 0.002	0.274 ±0.001	0.231 ±0.0026	0.389	0.0426*±0.0 0133
White panax ginseng root saponins	250	0.620 ± 0.0036	0.476 ±0.00216	0.335 ±0.0021	0.285 ±0.001	0.243 ± 0.001	0.337	0.0337± 0.00137
Control	2% saline (Vehicle)	0.701+ 0.0073	0.665 +0.000816	0.625 + 0.00368	0.456+0.00816	0.402 ± 0.0015	0.299	0.0152+0.00 23

N=5 Route of administration of carbon suspension – intracenous
8P<0.05 Student t-test

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