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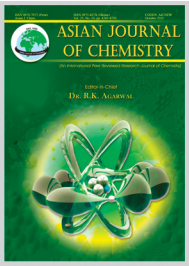

Extraction, Fractionation and Pharmacological Activity of *Dipteracanthus prostratus* (Poir) Nees

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Whole plant of *Dipteracanthus prostratus* (poir) nees were subjected to extraction and further fractionation to obtain isolated compounds. Different concentrations of methanolic extract were subjected to analgesic, antiinflammatory and wound healing assay by hotplate, paw oedema and excision wound model methods respectively. The methanolic extracts showed dose dependent analgesic, antiinflammatory and wound healing property as compared to standards. The extracts showed significant results in all three assays with increasing concentration. The phytochemical screenings confirm the presence of triterpenoids, flavonoids, alkaloids, phytosterols glycosides and carbohydrates, which are responsible for the analgesic, antiinflammatory and wound healing activity.

Key Words: *Dipteracanthus prostratus* (poir) nees, DPN-hexane-1, DPN-hexane-2, Analgesic, Antiinflammatory, Wound healing.

INTRODUCTION

Plants are the only economic source of a number of well established and important drugs. In addition, they are the source of same chemical intermediates needed for the production of a number of drugs. Our traditional system of medicine like Ayurveda, Siddha *etc.*, categorized so many plant species and their usage. Later on the allopathic system of medicine comes to force, due to the fast relieving nature it reached the world as quickly and diminished the usage of plant medicine as maximum. Even though natural products are on integral part of human health care system nowadays because there is now popular concern over toxicity and side effects of modern drugs[1,2]. The plant *Dipteracanthus prostratus* (poir) nees (DPN) (acanthaceae (ruellia family)) is a perennial herb, with stems often rooting at the nodes. It is widely distributed in listed as present in deciduous forests throughout India, Srilanka and East Africa[3]. The various part of the plant of DPN have been used traditionally for so many diseases like, anticancer against the epidermis of the nasopharynx region, slightly hypoglycemic, gonorrhoea and eye disease. A mixture of sterols containing stigmasterol (75.33), sitosterol (17.61), campesterol (7.04), cholesterol (trace) and brassicastrol (trace) isolated from leaves[4]. Hence, we have conducted this study to know phytochemical evaluation and pharmacological studies of methanolic extract of *Dipteracanthus prostratus* and isolate to the each components and study its characterization.

EXPERIMENTAL

The whole plant of *Dipteracanthus prostratus* (poir) nees (DPN) were collected during the month of August from Sathankulam, Tuticorin District, India and authentified by Mr. Chelladurai, Research Officer (Retired), Botany C.C.R.A.S. Government of India, Tirunelveli, India. The whole plants were dried under shade and made into powder form by using hand grinder mixer individually. Then the powder was passed from sieve no. 22 to get uniform powder. Physico-chemical values such as the percentage of total ash, acid insoluble ash, water soluble ash, sulphated ash, moisture content, petroleum ether, ethyl acetate, chloroform, ethanol and water soluble extractive values of DPN were calculated as per the Indian pharmacopoeia[5,6]. The extracts of whole plants of DPN were prepared by cold maceration with methanol. The whole part of the plant were dried under shade and coarsely powdered. About 1 kg of dried powder was macerated in methanol (3 × 1.5 L) for 14 days with occasional shaking, after completion of the extraction, the methanol extracts was filtered and concentrated at 55 ºC on water bath, till it acquires maximum concentrations. A dark green residue was obtained. This residue was used for fractionation by using various solvents like hexane, chloroform, ethyl acetate in gradient elution. Methanol extract and its fractions were subjected to analysis under ultra violet light. The methanol extract and its fractions were subjected to preli-

minary phytochemical screening for the detection of various chemical constituents. The presence or absence of different phytoconstituents *viz*. triterpenoids, steroids, alkaloids, sugar, tannins, glycosides and flavanoids, *etc*. were detected by usual prescribed methods[7].

Isolation of compounds: Wet packing technique used for the preparation of column packing. The adsorbent material, silica gel was mixed with solvent of non-polar and poured gently from the top of the column to a desire length then the same solvent was run through the column for 2-3 times to prevent air entrapment and the solvent used was maintained up to 10 cm above the column bed. Hexane fractions extracts (10 g) was dissolved in small amount of hexane and mixed thoroughly with silica gel and dried to have free flowing nature. The sample mixture was placed on the top of the column with the aid of funnel. The column was allowed to stand overnight. Then the column was eluted by gradient elution technique using different solvents with gradually increasing the polarity by changing the ratio of solvents (petroleum ether, hexane, chloroform, ethyl acetate and methanol). The flow rate of solvent system was adjusted between 16-20 drops per min. Each fraction was collected to maximum of 100 mL and it was evaporated at low temperature. Table-1 expressed the elution of hexane fraction by column chromatography. All fractions were routinely checked by TLC with Merck silica gel 60 G aluminium plates for the polysterol and triterpenoids identification. Melting point was determined on an open capillary tube method. The structural characterizations of isolated compounds were carried out by FTIR, ^{1}H NMR and Mass spectra. IR spectra were recorded on Shimadzu 8400 series Fourier transformer with KBr disks. ^{1}H NMR spectra were recorded in DMSO-d_6 on a JEOL spectrometer using TMS as an internal standard. Mass spectra were recorded on a Hewlett-Packard 1100 mass spectrometer.

TABLE-1
VARIOUS FRACTION OF COLUMN CHROMATOGRAPHY (HEXANE EXTRACT)

Solvents	Ratio (%)	Nature of residues
Petroleum ether	100	Dark green residue
Petroleum ether: Hexane	90:10	Green residue
Petroleum ether: Hexane	80:20	Green residue
Petroleum ether: Hexane	60:40	Yield is low
Petroleum ether: Hexane	40:20	Pale residue
Petroleum ether: Hexane	20:80	Low yield
Hexane	100	Brownish residue
Hexane: Chloroform	80:20	Light green residue
Hexane: Chloroform	60:40	Low yield
Hexane: Chloroform	40:60	Low yield
Hexane: Chloroform	20:80	Pale residue
Chloroform	100	Dark brownish residue
Chloroform: Ethyl acetate	95:5	Brownish residue
Chloroform: Ethyl acetate	90:10	Pale yellow powder
Chloroform: Ethyl acetate	80:20	Light brownish residue
Chloroform: Ethyl acetate	70:30	Light brownish residue
Chloroform: Ethyl acetate	60:40	Light brownish residue
Chloroform: Ethyl acetate	50:50	Colourless crystal
Chloroform: Ethyl acetate	40:60	Green residue
Chloroform: Ethyl acetate	30:70	Low yield
Chloroform: Ethyl acetate	20:80	Low yield
Chloroform: Ethyl acetate	10:90	Pale yellow

***In vivo* studies:** From reported toxicity study of DPN, 750 mg/kg i.p. as LD50 was selected[8]. Male albino mice (30-35 g) and male wistar rats (150-200 g) were fed with a standard diet and water *ad libitum*. The animals were housed in spacious polypropylene cages bedded with rice husk. The animal room was well ventilated and maintained under standard experimental conditions (temperature 27 ºC and 12 h light/dark cycle) throughout the experimental period. Animal experiments were carried out following the guidelines of the animal ethics committee of the institute.

Analgesic activity: Male albino mice were randomly divided in to 4 groups. (6 mice/group) and were administered by intraperitoneal route. Animals in-group **I** were treated aqueous Tween 80 (10 %) as control and the experimental rats are groups **II** animals were treated with pentazocine (5 mg/kg) and group **III** and **IV** animals treated with methanolic extract 250 mg/kg and 500 mg/kg respectively. Eddy's hot plate was maintained at constant temperature 55 ± 1 ºC and normal basal reaction time was recorded at 0, 30, 60, 90 and 120 min by observing jumping response on each animals.

Antiinflammatory activity: Male albino mice were randomly divided in to 4 groups. (6 mice/group) and were administered by intraperitoneal route. Animals in group **I** were treated with aqueous Tween 80 (10 %) as control and the experimental rats are groups **II** animals were treated with diclofenac sodium (10 mg/kg) and group **III** and **IV** animals treated with methanolic extract 250 mg/kg and 500 mg/kg, respectively. After 0.5 h of the administration, 0.1 mL of 1 % (w/v) carrageenan was injected in the plantar region of the left paw of control as well as drug treated animals, the right paw will serve as reference non inflamed paw for comparison. Paw volume of both legs was measured up to 4 h at 0.5 h interval.

Wound healing activity: Preparation of ointments containing 0.25 % w/w and 0.5 % w/w of methanolic extract were prepared by fusion method with 10 g of white petroleum jelly. In the excision wound model, the full-thickness excision wound were made on the rats by removing a 500 mm^2 piece of the skin from the depilated back after being anaesthetized with anaesthetic chloroform by the open-mask method. After skin excision, the wound was left open to the environment. Male wistar rats were randomly divided in to 4 groups. (6 rats/group) and were administered by intraperitoneal route. Animals in group **I** were treated with white petroleum jelly as control and the experimental rats are groups **II** animals were treated with standard soframycin ointment and group **III** and **IV** animals treated with 0.25 % and 0.5 % w/w of methanolic extract ointments respectively. Wound diameter was measured daily and note down.

Statistical analysis: All the analysis was done in triplicate and results are expressed as mean ± SD.

RESULTS AND DISCUSSION

The results of physicochemical studies obtained were 8.72 ± 0.016 for total ash, 3.65 ± 0.94 for water soluble ash, 0.822 ± 0.049 for acid insoluble ash, 9.51 ± 0.123 for sulphated ash and 10.27 ± 0.028 for moisture content. Petroleum ether, ethyl acetate, chloroform, ethanol and water soluble extractive values of DPN were 1.287 ± 0.172, 5.159 ± 0.03, 7.249 ± 0.103,

10.474 ± 1.098, 6.231 ± 0.964 respectively. After cold maceration, dark green residue was obtained for methanol extract and its fraction having dark green, green and brown for hexane, chloroform and ethyl acetate respectively under day light. Green flouresence obtained under UV light for methanolic extract and its hexane and chloroform fraction. Brown fluorescence obtained under UV light for ethyl acetate fraction of methanolic extract of DPN. The phytochemical screening was proved that carbohydrate and flavonoids were present in methanolic extract and its chloroform and ethyl acetate fractions, alkaloids and phytosterols were found in methanolic extract and its ethyl acetate fractions, glycoside were found in methnolic extract and its all fraction except ethyl acetate, phenolic compound (tannins) and phytosterols were present in hexane fraction, triterpene and phenolic compound (tannins) were present in methanolic extract and its hexane fraction. Hexane fraction of methanolic extract of air dried whole plant of DPN were selected and eluted by different ratio of solvents with increasing polarity and collected fractions were undergone for investigation by TLC. Chloroform and ethyl acetate fractions of 50:50 and 90:10 have shown a spot in the mentioned mobile phase. Those spots produced fractions were selected and evaporated for isolation of compound. Compound DPN-hexane-2 and compound DPN-hexane-1 were isolated and purified from chloroform and ethylacetate fractions 50:50 and 90:10, respectively. Compound DPN-hexane-2 was isolated as described above to obtain a colourless crystals; m.p. 145 ºC; R_f = 0.235 (Ethyl acetate(**4**) : Chloroform(**1**) using 50 % sulphuric acid as a detecting agent); Soluble in chloroform, dimethylsulfoxide and methanol; FTIR spectra wave numbers 3406 cm^{-1} (broad peak for hydrogen bonding OH stretching), 2959 and 2880 cm^{-1} (for C-H stretch for CH_2 and CH_3), 1465 cm^{-1}(CH bending for CH_2), 1368 and 1255 cm^{-1} (for C-H bending for dimethyl), 1074 and1024 cm^{-1} (for C-O strech). ^{1}H NMR (δ/ ppm) 0.9-1.3 (s, 18H), 1.4 (s, 6H), 1.5-2.0 (s, 18H), 2.2 (m, 1H), 2.4 (m, 4H), 3.5 (s, 1H), 4.2 (m, 1H), 5.32 (m, 1H); MS (EI): m/z = 414.27 [M^+]. Anal. Calcd. for $C_{29}H_{50}O$. Compound DPN-hexane-1 was isolated as described above to obtain a Pale yellow crystals; mp 274 ºC; Rf = 0.250 (Chloroform(**3**): ethyl acetate(**2**) using libermann - burchard reagent as a detecting agent); soluble in chloroform, dimethylsulfoxide and methanol; sparingly soluble in acetone; insoluble in water and petroleum ether; FTIR spectra wave numbers 3496 cm^{-1} (for OH stretching), 1620 cm^{-1}(for C=O stretch), 1446 cm^{-1}(CH bending for CH_2), 1325 and 1300 cm^{-1} (for C-H bending for dimethyl) and 1150 cm^{-1} (for C-O strech). ^{1}H NMR (δ/ ppm) 0.7 (s,3H), 0.8 (s, 3H,), 0.9 (s, 3H), 1.10 (s, 6H), 1.2-1.4 (s, 6H), 1.5 (s, 6H), 1.7-1.8 (s, 18H), 3.1 (m, 1H), 5.2 (w, 1H), 12.0 (w, 1H); MS (EI): m/z = 456.25 [M^+]. Anal. Calcd. for $C_{30}H_{48}O_3$.

In analgesic studies, prominent effect of methanolic extract of whole plant of DPN showed a highly significant analgesic activity ($p < 0.01$)at the both dose of 250 and 500 mg/kg employed in 0.5 h after inducing the heat (55 ºC) by using hot plate method when compared with standard pentazocine (5 mg/kg). After 0.5 h, there is a gradual reduce in analgesic activity in both methanolic extract doses and standard dose. The results were present in Table-2.

ABLE-2
IN VIVO ANAGLESIC ACTIVITY OF METHANOLIC EXTRACT OF *Dipteracanthus prostratus*

Treatment and dose (Per Kg)	Reaction time (s) (Mean ± SEM)				
	0 h	0.5 h	1 h	1.5 h	2 h
Tween 80 (10 %)	3.20± 0.38	2.31± 0.34	4.00± 0.26	3.68± 0.25	2.62± 0.22
Pentazocine (5 mg/kg)	4.22± 0.82	14.5± 0.96**	11.3± 0.48**	10.6± 0.24**	9.40± 0.18**
Methanolic Extract (250 mg)	4.09± 0.02	6.68± 1.02**	5.30± 0.74*	4.32± 1.08	3.02± 0.51

(Mean ± SD); n = 6; *p < 0.1; **p < 0.01

In this study, prominent effect of methanolic extract of whole plant of DPN showed a significant antiinflammatory activity at the dose of 250 and 500 mg/kg. The carrageenan induced rat paw oedema test showed a close related anti inflammatory activity in the extracts treated animals. A significant decrease in the oedema was observed in 1.5 h at doses of 250 and 500 mg/kg. In 2.5 h highly significant decrease in the oedema was observed at a dose of 250 and 500 mg/kg which is compared with the standard drug diclofenac sodium. The results were present in Table-3.

TABLE-3
IN VIVO ANTI INFLAMMATORY ACTIVITY OF METHANOLIC EXTRACT OF *Dipteracanthus prostratus*

Paw volume (mL) at (h)		Tween 80 (10 %)	Diclofenac sodium (10 mg/kg)	Methanol extract (250 mg/kg)	Methanol extract (500 mg/kg)
0.5	R	0.15±0.0089	0.13±0.044	0.13±0.020	0.16±0.020
	L	0.16±0.020	0.21±0.016	0.25±0.34	0.30±0.036
1	R	0.15±0.0089	0.13±0.044	0.13±0.020	0.16±0.020
	L	0.16±0.020	0.20±0.025	0.23±0.042	0.28±0.007
1.5	R	0.15±0.0089	0.13±0.044	0.13±0.020	0.16±0.020
	L	0.23±0.021	0.16±0.021	0.21±0.029	0.26±0.030
2	R	0.15±0.0089	0.13±0.044	0.13±0.020	0.16±0.020
	L	0.26±0.020	0.16±0.021	0.20±0.036	0.23±0.041
2.5	R	0.15±0.0089	0.13±0.044	0.13±0.020	0.16±0.020
	L	0.26±0.020	0.15±0.02**	0.20±0.036*	0.19±0.043*
3	R	0.15±0.0089	0.13±0.044	0.13±0.020	0.16±0.020
	L	0.28±0.030	0.15±0.02**	0.18±0.028*	0.17±0.028*
3.5	R	0.15±0.0089	0.13±0.044	0.13±0.020	0.16±0.020
	L	0.26±0.020	0.13±0.021	0.13±0.026	0.17±0.025
4	R	0.15±0.0089	0.13±0.044	0.13±0.020	0.16±0.020
	L	0.28±0.020	0.15±0.022	0.18±0.025	0.17±0.027

(Mean ± SD); n = 6; *p < 0.1; **p < 0.01

Methanolic extracts promoted the wound healing activity significantly in excision wound models studied. Studies reported that high rate of wound contraction will decrease in the period of epithelialization. After 14 days, methanolic extracts decreased the wound contraction rate dose dependent manner. 0.5 %w/w of methanolic extracts of DPN ointment has shown high significant wound healing activity compared with standard soframycin ointment and 0.25 %w/w methanolic extract of DPN. The results were present in Table-4.

Conclusion:

Finally we concluded that the methanolic extracts of DPN showed dose dependent analgesic, antiinflammatory and wound healing activity. Compounds like DPN-hexane-1 and

DPN-hexane-2 were isolated from hexane fraction of methanolic extracts and further studies are required to elucidate the structure of the two compounds.

TABLE-4
In vivo WOUND HEALING ACTIVITY OF METHANOLIC EXTRACT OF *Dipteracanthus prostratus*

	Days	Tween 80 (10 %)	Safromycin oinment	Methanolic extract (0.25 % w/w)	Methanolic extract (0.50 % w/w)
Diameter of the wound (cm)	0 (A)	2.1±0.17	2±0.15	2.1±0.17	2.4±0.19
	2	2.1±0.17	1.9±0.09	2±0.14	2.3±0.1
	4	2±0.14	1.8±0.2	1.9±0.28	2.1±0.09
	6	1.9±0.13	1.6±0.32	1.7±0.42	2±0.18
	8	1.9±0.13	1.4±0.28	1.6±0.12	1.8±0.21
	10	1.8±0.1	1.2±0.08	1.5±0.28	1.5±0.09
	12	1.7±0.09	1.1±0.24	1.3±0.13	1.3±0.1
	14 (B)	1.7±0.09	0.9±0.31	1.2±0.34	1.2±0.12
	A-B	0.4±0.08	1.1±0.21	0.9±0.08**	1.2±0.18*

(Mean ± SD); n = 6; *p < 0.1; **p < 0.01

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