J. Phytol. Res. 20(1): 133-135, 2007

PHYTOCHEMICAL AND PHARMACOLOGICAL INVESTIGATION OF *PHYLLANTHUS NIRURI* - A PRELIMINARY SCREENING

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Phyllanthus niruri powder was packed in the Soxhlet apparatus in the ratio of 1:4 with various solvents and the extract was subjected to various biochemical tests. Later the extract was tested for steroids, flavonoids, lactones, glycoside etc. The extract components were separated by TLC and further purified by column chromatography. Pharmacological investigation was carried out using normal rats and alloxan rats.

Keywords : Extracts; Pharmacological; Phytochemistry; Solvents; Soxhlet apparatus; TLC.

Introduction

About 2200 genera of nearly 386 families of medicinal plants mainly belonging to families such as Asteraceae, Euphorbiaceae, Laminaceae, Fabaceae, Rubiaceae, Poaceae, Acanthaceae, Rosaceae and Apiaceae. The genus Phyllanthus (is a Spanish name "Chanca piedra" means "Stone breaker" or "Shatter stone") belongs to Euphorbiaceae which contains over 600 species of shrubs and trees, and spread throughout the Tropical and Subtropical regions.

P.niruri is used as analgesic, antibacterial, antihepatotoxic, anti-inflammatory, antilithic, antimalarial, antimutagenic, antispasmodic, antiviral, carminative, chlolerectic etc. Similarly, Nara *et al.*[1] reported that *P.madaraspatensis* is reported to be carinative, laxative, antigent, diuretic and used in the treatment of bronchitis.

Ottow and Johres[2] was first to isolate a toxic bitter oricinole which was named Phyllanthin and assigned $C_{39}H_{37}O_8$. Chauhan *et al.*[3] extracted two glycoflavanes such as Kaempferol 4 –rhamnopyranoside and oridictory-7-rhamnopyranoside from *P. niruri* by using ethyl acetate solvent. Ahmed *et al.*[4] examined the seed of *P.niruri* and reported the presence of 1.2% ricinoleic acid. Since *P.niruri* is recommended as herbal remedy for few diseases, the present investigation was concentrated in the phytochemistry study of *P.niruri* by extraction methods and pharmacological investigation using Albino rats was carried out.

Materials and Methods

P.niruri was collected from a open field and surface sterilized before use. The plant was air dried for 10 days under shadow and powdered using matrix waring blender and sieved (No.5) and stored in air tight container. 100g of the plant powder was packed into the thimble of a Soxhlet apparatus in the ratio of 1:4 (powder:solvent) with different solvents such as petroleum ether, chloroform, methanol and ethyl acetate. After 16 h of distillation the extracts were Vacuum dried and stored in air tight container for further qualitative and biochemical tests.

The extract was subjected to various test for the presence of steroids, diterpenes, flavonoids, lactones, glycoside etc. The extraction compound was separated by Thin Layer chromatography and Rf value were calculated. Further purified by column chromatography and the samples were collected and quantified . Later pharmacological (hypoglycemic activity and blood sugar) investigation with albino rats and Alloxan induced rats were carried out as a clinical trial.

Results and Discussion

The leaf powder of *P.niruri* (100g) was refluxed serially using various solvent (petroleum ether, chloroform, methanol and ethyl acetate). The extracts of various solvents were concentrated and the physical and chemical characterization of the extracts are tabulated (Table.1). Row *et al.*[5], extracted bluish green essential oil from the leaves of *P.carcovadensis* which constituted Cymol-11,1-limonen upon steam distillation.

The extracts of each solvent were subjected to various chemical tests for steroids, diterpenes, alkoids, flavonoids, lactones and glycosides and the results are tabulated in Table 2. Among the solvents, ethyl acetate extract showed positive test for glycosides by Molisch's test (formation of brown ring between two layers).

The extracts of each solvent were subjected to TLC. Among the four extracts, methanol and chloroform does not give any proper response in qualitative test and TLC. Ethyl acetate extract showed three of pale green, reddish yellow and dark green at the ratio of 2:6:2 of ethyl

Table 1. Physical examination and characterization of the separated extracts.

Extracts	Dry Weight	Colour	Consistency	Odour
1. Petroleum Ether	1.07g	Yellowish Green	Sticky	No characteristic odour
2. Chloroform	0.78g	Green	Sticky	No characteristic odour
3. Methanol	0.67g	Reddish green	Sticky	No characteristic odour
4. Ethyl Acetate	1.06g	Blackish green	Sticky	No characteristic odour

Table 2. Result for qualitative test.

S.No.	Chemical Test	Extracts			
		Petroleum ether	Chloroform	Methanol	Ethyl acetate
1.	Test for steroids				
	a) Salkaowski test	+	-	-	-
	b) Libermann-burchard test	+	-	-	-
2.	Test for Diterpenes				
	a) Cupric Acetate test	-	-	-	-
3.	Test for Alkaloids				
	a) Mayer's test	-	-	-	-
	b) Wagner's test	-	-	-	-
	c) Hager's test	-	-	-	-
	d) Dragndorffs test	-	-	-	-
4.	Test for Flavonoids				
	a) Ferric Chloride test	-	-	-	-
	b) Shimoda test	-	-	-	-
	c) Zinc-HCL-reduction test	-	-	-	-
	d) Lead acetate test	-	-	-	-
5.	Test for Lactones				
	a) Legal's test	-	-	-	-
	b) Feigel's test	-	-	-	-
6.	Test for Glycosides				
	a) Molisch's test	-	-	-	+

Table 3. Effect of crude compound on normal rats.

Rats No.	Weight of rats in gram	Blood sugar level in mg/100ml				Percentage reduction at the end of 3rd hour
		0 hour	1 hour	3rd hour	5th hour	
Control 1	165	60.71	62.50	60.71	64.28	–
Control 2	172	67.85	67.85	71.42	78.03	–
Drug Injected rat1 (D1)	191	69.64	67.55	64.07	66.16	7.99
Drug Injected rat 2(D2)	180	66.07	63.2	60.12	62.10	9.00

Table 4. Effect of crude compound on Alloxanised rats.

Rats No.	Weight of rats in gram	Blood sugar level in mg/100ml				Percentage reduction at the end of 3rd hour
		0 hour	1 hour	3rd hour	5th hour	
Control 1	175	187.5	210.71	241.07	253.57	-
Control 2	190	217.85	225.00	221.42	239.28	-
Test rat+ Drug	195	164.28	148.21	132.14	142.85	19.56
Test rat+ Drug	167	178.57	160.71	139.28	128.57	22.00

acetate: chloroform: methanol solvent with different Rf values of 0.44, 0.73 and 0.93, respectively. The phytochemical examination of ethyl acetate extract of *P.niruri* roots resulted in two glycoflavanes such as Kaempferol-4' rhamnopyranside and oriodictory-7-rhamno pyranoside[6].

Petroleum ether extract gave one clear and separate band at the ratio of 7: 2 : 1 of water : ethanol : ethyl acetate solvent with a Rf value of 0.47. The petrol extract of the stem and leaves of *P.rediculatus* on chromatography gave in succession friedelin, glochionol, 2,1,alpha-hydroxy friedelin having terminal double bond[7].

The ethyl acetate extract was purified by column chromatography. The reddish yellow colour compound showed positive result for glycosides. Therefore the reddish extract was subjected to spectral studies and it was found to have higher absorbance at 470 nm due to the presence of phenolic and carbonyl compounds.

In pharmacological investigation, four male albino rats weighing 150 g-200g were fasted for 24 h and they were used as experimental animals. Among them two rats were considered as control and two others as Alloxan induced rats. About 100 mg/Kg weight of petroleum ether was injected in the present study for estimation of blood sugar level at regular intervals and results are tabulated in the Table 3-4. The glucose level was decreased significantly about 80-120 mg/ml blood when compared to normal animals.

The present investigation, is a prime report and it needs further establishment of screening in terms of medicinal value through clinical trials and structural elucidation of compounds in future.

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