



Phytochemical and Anthelmintics Activity of *Phyllanthus reticulatus* (Poir)

Mythili A*

Department of Pharmaceutical Chemistry, Vivekanandha Pharmacy College for Women, Sankari Tamilnadu, India.

Received: 16 Oct 2019 / Accepted: 14 Nov 2019 / Published online: 01 Jan 2020

*Corresponding Author Email: mythilisatheesh22@gmail.com

Abstract

Aim and objective: *Phyllanthus reticulatus* poir is used to treat many diseases and used as antidiabetic, antiplasmodial activity, Hypocholesterolemic activity, Antimicrobials, Cytotoxic activity, Hepatoprotective activity., etc. The present study was aimed to separate phytochemicals present in it and analysis of its anthelmintic activity. **Materials and methods:** *Phyllanthus reticulatus* poir leaves are shade dried and coarsely Powdered plant leaves were used for the extraction purposes. It was extracted with various solvents such as petroleum ether, chloroform, ethyl acetate and ethanol. Extracted materials were subjected to phytochemical analysis and to found anthelmintic activity of it against *Peritima posthuma*. **Results and discussion:** Phytochemical screening of the extracts revealed the presence of Carbohydrates, Proteins, Phytosterols, Alkaloids, Saponins, Flavonoids, Terpenoids, Tannins and Steroids. It was found that Ethanolic and Aqueous extract exhibited anthelmintic activity in dose dependent manner Giving the shortest time of paralysis and death with 60mg/ml concentration. **Conclusion:** The ethyl acetate and alcoholic extract of *Phyllanthus reticulatus* poir Wight & Arm not only demonstrated paralysis, but also caused death of worms especially at higher concentration of 60 mg per ml is shorter time as compared to that reference drug Albendazole. The data suggested that the Aqueous extract has more significant anthelmintic activity then ethanolic extract.

Keywords

Anthelmintic activity, *Peritima posthuma*, *Phyllanthus reticulatus* (poir), Phytochemicals.

INTRODUCTION

In our traditional culture millions of medicinal plants used all over the world, particularly nowadays natural plants used to treat cure diseases instead of synthetic medicines. *phyllanthus reticulatus* poir is medicinal plant which is belongs to Euphorbiaceous family and it is used to treat many diseases and uses as antidiabetic, antiplasmodial activity, Hypocholesterolemic activity, Antimicrobials, Cytotoxic

activity, Hepatoprotective activity, Antibacterial activity, Antinociceptive and Anti-hyperglycemic activity, Analgesic and Anti-inflammatory activity, Antioxidant properties.

Leaves of *phyllanthus reticulatus* poir contains tannic acid, friedelin, epifriedelinol, betulin, taraxerone, beta - sitosterol, glochidonol, octacosanol, taraxeryl acetate and 21 - alpha - hydroxyfriedelan - 3 - one, betulinic acid. Stem bark contains contains coumarin

eight compounds (β - sitosterol - 3 - O - β - glucoside, stigmasterol - 3- O- β - glucoside, methyl brevifolin carboxylate, kaempferol, astragalol, methyl gallate, ellagic acid, corilagin including two flavonoid) glycoside rutin (quercetin - 3 - rutinoside) and quercetin - 3 - O - β - D glucopyranoside. (Shalini Sharma et al., 2013)

MATERIALS AND METHODS

Collection

The plant *phyllanthus reticulatus* poir belonging to family Euphorbiaceae are grown widely throughout India. The plants were collected from Bhavani, Erode district, Tamilnadu, India in the month of April 2016.

Preparation of extract:

The collected, cleaned and coarsely powdered leaves of *phyllanthus reticulatus* (120gm) were used for the extraction purposes. It was then extracted with various solvents such as petroleum ether, chloroform, ethyl acetate and ethanol. The extraction was carried out with solvents by soxhlet extraction for 48 Hrs. After each solvent extraction, the extracts were filtered through whattmann filter paper to remove any impurities is present (Kokate, 1994).

a) Petroleum ether extraction of leaves of *Phyllanthus reticulatus*:

About 120 gm of dry coarse powder was extracted first with Petroleum ether (60-80°C) in a hot soxhlet extraction using round bottomed flask for 48 Hrs. After completion of extraction, the product mass was filtered and the excess of solvent was removed by distillation under reduced pressure. A green colure residue was obtained. The extract was then stored in a desiccator (Kokate, 1994).

b) Chloroform extraction of leaves of *Phyllanthus reticulatus*:

The marc left after Petroleum ether extract was dried and subsequently extracted with chloroform (40-60°C) in a hot soxhlet extraction using round bottomed flask for 48 Hrs. After completion of extraction, the product mass was filtered and the excess of solvent was removed by distillation under reduced pressure. A green colour residue was obtained. The extract was then stored in a desiccator (Kokate, 1994).

c) Ethyl acetate extraction of leaves of *Phyllanthus reticulatus*:

The marc left after Chloroform extract was dried and subsequently extracted with ethyl acetate (40-60°C) in a hot soxhlet extraction using round bottomed flask for 48 Hrs. After completion of extraction, the product mass was filtered and the excess of solvent was removed by distillation under reduced pressure.

A green colour residue was obtained. The extract was then stored in a desiccator (Kokate, 1994).

d) Ethanolic extraction of leaves of *Phyllanthus reticulatus*:

The marc left after Ethyl acetate extract, was dried subsequently extracted with ethanol (40-60°C) in a hot soxhlet extraction using round bottomed flask for 48 Hrs. After completion of extraction, the product mass was filtered and the excess of solvent was removed by distillation under reduced pressure. A green colour residue was obtained. The extract was then stored in a desiccator (Kokate, 1994).

DETECTION OF PHYTOCHEMICALS

The different extracts obtained in extraction process were analyzed systematically for the different chemical constituents to assess the active constituent present and the nature of polarity of the constituents like alkaloids, carbohydrates, steroids, fixed oil, fats, tannins, phenolic compounds, proteins, amino acids, saponins, gums, mucilage's, flavonoids etc., (IP., 1996)

Detection of Carbohydrates:

Molisch's test:

The extracts are treated with 2-3 drops of 1%alcoholic α -naphthol and 2ml of concentrated Sulphuric acid was added along with the sides of the test tube. Carbohydrate is indicated by the appearance of purple ring between junctions of two liquid layers.

Fehling's test:

The extracts are treated with Fehling's A and B solution and heated for few minutes. The red precipitates formed indicate the presence of reducing sugar.

Benedict's test:

The extracts are treated with Benedict's reagent and heated for few minutes. The red precipitate indicates the presence of reducing sugar.

Detection of Glycosides:

Minimum quantities of the extracts were hydrolyzed with hydrochloric acid for few minutes on water bath and the hydrolysate is subjected to the following tests.

Legal's test

To the hydrolysate 1ml of pyridine and few ml of sodium nitroprusside solution were added to this sample and then it was made alkaline with sodium hydroxide solution. Color change indicates the presence of glycosides.

Borntreger's test

Chloroform is treated with hydrolysate and the chloroform layer is separated. In this chloroform layer, dilute ammonia solution equal quantity is added. Color change in the ammoniacal layer shows the presence of glycosides.

Detection of Proteins and Amino Acids

Small quantities of the extracts were subjected to the following tests.

Million's test

The extract were treated with million's reagent. A red color produced and it indicated the presence of proteins.

Ninhydrin test

The extract were treated with ninhydrin reagent. Purple color indicated the presence of protein.

Biuret test

The extract were treated with equal volume of 5% sodium hydroxide solution and 1% copper sulphate solution. A violet colour produced and it indicated the presence of proteins.

Detection of Fixed Oils and Fats:

Spot test

Small quantities of the extracts were placed between two filter papers. Oil stains produced with any extracts shows the presence of fats and fixed oils in the extracts.

Saponification test

Few drops of 0.5N alcoholic potassium hydroxide added to the extracts with few drops of phenolphthalein solution. Later the mixture is heated on water bath for about 1-2 hrs., soap formation indicates the presence of fat and fixed oils in the extracts.

Detection of Gums and Mucilage:

Ruthenium Red Test:

Small quantities of the extracts were diluted with water and ruthenium red solution was added. A pink color indicates that presence of gum and mucilage.

Detection of Alkaloids:

Mayer's test:

To about 3 ml of sample, add few drops of Mayer's reagent. Precipitate is formed and it indicates that presence of alkaloid.

2. Dragendorff's test:

To about 3 ml of sample, a few drops of Dragendorff's reagent are added. Brownish fluorescent precipitate is formed and it indicates that presence of alkaloid.

3. Wagner's test:

To about 3 ml of sample add few drops of Wagner's reagent. Brownish precipitate is formed and it indicates that presence of alkaloid.

4. Marme's test:

To about 3 ml of extract, add few drops of Marme's reagent. Then dil. H₂SO₄ is added to the sample mixture. White or yellow precipitate is formed and precipitate was dissolved on excess addition of Marme's reagent or ethanol and it indicates that presence of alkaloid.

PRELIMINARY PHYTOCHEMICAL STUDIES

Preliminary phytochemical studies of the Leaves of *P. reticulatus* poir:

The preliminary phytochemical studies were done in the extracts of the leaves of *P. reticulatus* poir was done for the presence of Carbohydrate, Glycosides, Proteins, Fixed oils & Fats, Flavonoid's, Gums & mucilage, Phytosterol, saponins and Alkaloids were present and It were shown in the Table.no. 1

S.no	Phytochemical test	Extract			
		Pet.Ether	Ethylacetate	Ethanol	Water
Carbohydrate:					
1	Molisch's Test	+	+	+	+
	Fehling's Test	+	+	+	+
Proteins & Aminoacids:					
2	Million's Test	+	-	-	+
	Biuret Test	-	-	-	+
	Ninhydrin Test	-	-	-	+
	Xanthoprotic Test	-	-	-	+
Saponins:					
3	Frothing Test	-	+	+	+
	Heamolysis test	-	+	+	+
Phytosterols:					
4	Libermann's test	-	+	+	+
	Salkowski's test	+	+	-	-
Alkaloids:					
5	Mayer's test	-	-	+	+

	Dragendroff's test	+	-	+	+
	Hager's test	-	-	+	+
	Wagner's test	+	-	+	+
	Flavonoids:				
6	NaOH test	+	+	+	+
	H ₂ SO ₄ test	-	+	+	+
	Shinoda's test	-	+	+	+
	Phenolic compounds:				
	Bromine water	-	+	+	+
7	Acetic acid solution	-	+	+	+
	Dilute iodine solution	-	+	+	+
	Dilute HNO ₃	-	+	+	+
	Tannins				
8	5% FeCl ₃ solution	-	+	+	+
	Lead acetate solution	-	+	+	+
	Gelatin solution	-	+	+	+
	Triterpinoids				
9	Salkowski test	+	+	+	-

(+) indicates presence, (-) indicates absence

Table No. 1: Qualitative phytochemical analysis of *P.reticulatus*.

PHARMACOLOGICAL ACTIVITY

Anthelmintic activity:

It was found that Ethanolic and Aqueous extract exhibited anthelmintic activity in dose dependent manner giving the shortest time of paralysis and death with 60mg/ml concentration. The Aqueous extract of *Phyllanthus reticulatus* poir Wight & Arm caused paralysis in 20.05 min and time of death in 27.69 min while Ethanolic extract caused paralysis and death in 13.77 and 20.75 min respectively against the earthworm *Peritima posthuma*. The standard drug Albendazole showed the same at 17.86 min and 25.80 min respectively.

Albendazole by blocking glucose uptake and depletion of glycogen stores in the parasite exhibits

anthelmintic activity. The ethyl acetate and alcoholic extract of *Phyllanthus reticulatus* POIR Wight & Arm not only demonstrated paralysis, but also caused death of worms especially at higher concentration of 60 mg per ml is shorter time. Chemically tannins are polyphenolic compounds. It is possible that tannins contained in the extracts of *Phyllanthus reticulatus* POIR Wight & Arm produce similar effects of tannins it can bind to free proteins in host animal of the gastrointestinal tract or parasites glycoprotein on the cuticle and may cause death. (S.Athnasiadou et al., 2001)

Anthelmintic activity of Leaves of *Phyllanthus reticulatus* poir Wight & Arm and standard values were tabulated as follows

S.No	Treatment	Dose (mg/ml)	Time For Paralysis(min)	Time For Death (min)
1	Control	----	----	----
2	Standard	20	26.97 ± 0.55	36.47 ± 0.80
3	Standard	40	22.31 ± 0.33	31.79 ± 0.26
4	Standard	60	17.86 ± 0.32	25.80 ± 0.26
5	Aqueous Extract	20	20.05 ± 0.24*	27.69 ± 0.23*
6	Aqueous Extract	40	16.17 ± 0.21*	23.78 ± 0.22*
7	Aqueous Extract	60	12.05 ± 0.23*	19.00 ± 0.19*
8	Ethanolic Extract	20	21.81 ± 0.33*	30.15 ± 0.42*
9	Ethanolic Extract	40	18.15 ± 0.24*	24.74 ± 0.31*
10	Ethanolic Extract	60	13.77 ± 0.26*	20.75 ± 0.31*

Table no 2: Anthelmintic activity (death time) of Leaves of *Phyllanthus reticulatus* poir Wight & Arm and standard

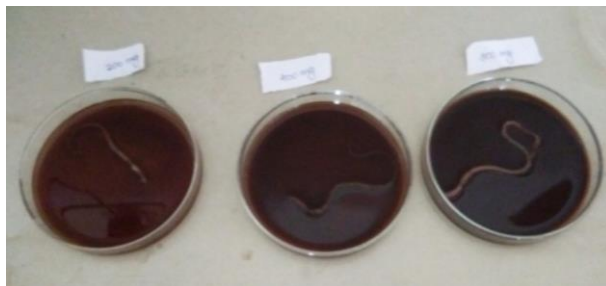


Figure no- 1: Aqueous extract of *Phyllanthus reticulatus* POIR with peritima posthuma at initial stage



Figure no- 2: Aqueous extract of *Phyllanthus reticulatus* POIR with peritima posthuma at death stage



Figure no- 3: Ethanolic extract of *Phyllanthus reticulatus* POIR

STATISTICAL ANALYSIS OF ANTHELMINTIC ACTIVITY

Each value represents mean \pm S.E (n=6) and was analyzed by ANOVA Turkey-Kramer multiple comparison test $*p < 0.001$. From that chart, Death

time of worm was denoted in Y axis and concentration of standard and sample extract were denoted in X axis.

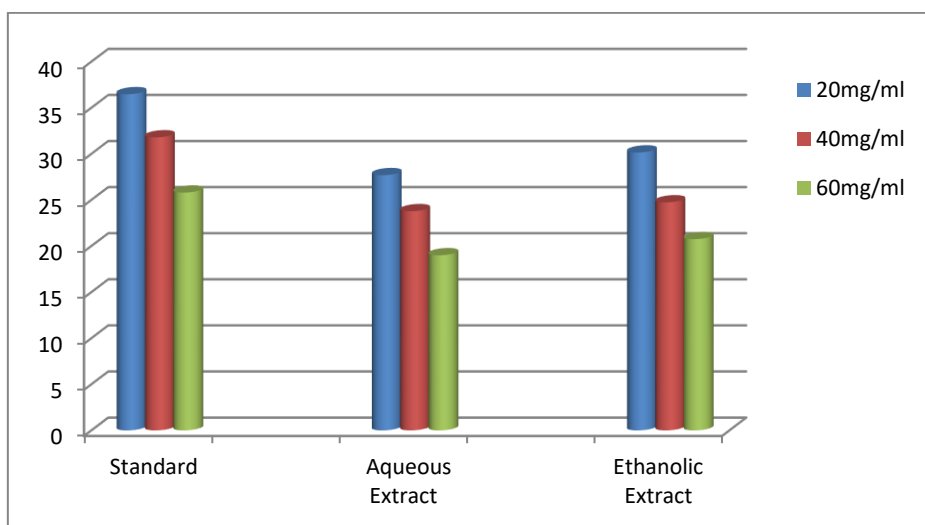


Chart No-1: Comparison of Death time of peritima posthuma treated with extract *Phyllanthus reticulatus* POIR Wight & Arm and standard drug albandazole

RESULT AND DISCUSSION

The powdered leaves of *phyllanthus reticulatus* poir belonging to the family Euphorbiaceae have been investigated in a systemic way covering from successive extraction, Preliminary phytochemical study to analyze the active constituent of the plant. Aqueous extract, Ethanolic extract of the leaves of the plant was subjected to anthelmintic activity. Control drug didn't showed any paralysis activity. A standard drug albandazole was subjected to peritima posthuma according to 20 mg/ ml, 40 mg/ ml and 60 mg/ ml concentrations. Paralysis, death time was noted, 20 mg/ ml of drug concentration showed paralysis and death time were 26.97 ± 0.55 and 36.47 ± 0.80 this concentration took more time to kill peritima posthuma meanwhile 60 mg/ ml of drug concentration of drug was kill peritima posthuma in short time like 17.86 ± 0.32 and 25.80 ± 0.26 . After that an Aqueous and ethanolic extract were treated with earth warm and the concentrations like 20 mg/ml, 40 mg/ml, 60 mg/ml. Compared to low concentration, a high concentration of extract showed paralysis and kill the earth warm very shortly. 60 mg/ml of aqueous extract showed paralysis, death time were $12.05 \pm 0.23^*$ and $19.00 \pm 0.19^*$. 60 mg/ml of ethanolic extract showed paralysis, death time against peritima posthuma were $13.77 \pm 0.26^*$ and $20.75 \pm 0.31^*$

CONCLUSION

Phytochemical investigation of Aqueous extract and Ethanolic extract of leaves of *Phyllanthus reticulatus* POIR Wight & arm showed the presence of carbohydrates, proteins and Amino acids, saponis, ptosterol, Alkaloids, Flavonoid's, phenolic compounds and Tannins.

The Aqueous extract and ethanolic extract of leaves of *phyllanthus reticulatus* poir Wight & arm showed

significant Anthelmintic activity in dose dependent manner giving shortst time of paralysis and death with 60 mg/ml concentration in Earth warm (*peritima posthuma*). The data suggested that the Aqueous extract has more significant anthelmintic activity then ethanolic extract. If other further related studies and clinical trials are carried out it will definitely open up a new vista in modern medicine. The present study could be concluded in a very clear cut manner from the investigation that *phyllanthus reticulatus* poir Wight & Arm of family Euphorbiaceae showed effective in Anthelmintic activity.

ACKNOWLEDGEMENTS

Our special thanks to **Prof.Dr.M.Karunanithi**, B.Pharm, M.S., Ph.D, D.Litt, chairman & Secretary of Vivekanandha educational institutions for providing infrastructures and facilities required for this research work.

Our special thanks to **Dr.P.Ashok kumar**, M.Pharm., principal., vivekanandha college of pharmacy for providing infrastructures and facilities required for this research work.

REFERENCE

1. Shalinisharma and Sunil Kumar, IJPSR, June, 2013: vol. 4(7): 2528-2534.
2. Indian Pharmacopeia, 1996, vol-2, A54, 3rd edition.
3. Kokate C K, Textbook of pharmacognosy (4th Edn) Vallabah Prakashan, New Delhi, 1994, pp 112-120.
4. WWW. Siddhainfo.com-phyllanthus reticulatus.
5. Indian Journal of natural products, 2002, 18(2), 8-9.
6. Athanasiadou S, Kyriazakis I, Jackson F and Coop RL, Direct helmentic effects of condensed tannins towards different gastrointestinal nematodes of sheep: *in vitro* and *in vivo* studies. *Vet. Parasitol*, 2001, 99: 205-19.