WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.084
Volume 11, Issue 3, 9-15. Research Article ISSN 2277– 7105

THIN LAYER CHROMATOGRAPHY AND PHYTOCHEMICAL SCREENING OF LEAVES EXTRACT OF *PHYLLANTHUS RETICULATUS* POIR. (FAMILY: EUPHORBIACEAE)

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Article Received on
26 December 2021,

Revised on 15 Jan. 2022,
Accepted on 04 Feb. 2022

DOI: 10.20959/wjpr20223-22394

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ABSTRACT

Phyllanthus reticulatus Poir. is a medicinal plant belongs to the family Euphorbiaceae which is widely found in asiatic region. This plant has been reported to have important phytoconstituents and used for medicinal activities like antidiabetic, antiviral, anticancer, antiplasmodial, hepatoprotective, antibacterial and anti-inflammatory activities for long times. The current study was carried out to investigate the phytochemicals present in the methanolic leaf extracts of *Phyllanthus reticulatus* Poir. and to calculate the Rf value of compounds found by thin layer chromatography. The dried powders were extracted by maceration process in methanol 90% methanol in water. The phytochemical screening reports that the plant contains phytochemicals like tannins, saponins, alkaloids, coumarins, terpenoids and flavonoids. The methanol fraction of the leaf extract was investigated by Thin Layer Chromatography (TLC) in different solvent system and three spots were found with the solvent system, Petroleum ether: Methanol (75: 25). R_f value of those spots were 0.40, 0.51 and 0.62. The plant has principles that might have good prospects in drug discovery from natural sources. However further investigations are necessary to check the findings.

KEYWORDS: *Phyllanthus reticulatus*, Phytochemical screening, Thin Layer Chromatography.

INTRODUCTION

Medicinal plants are used in the treatment of various diseases from ancient time. These plants contain different phytochemicals which are responsible to cure the specific diseases of the body. Phytochemicals are chemical constituents of plants and are used for as therapeutic agents. The use of herbs to cure disease is almost worldwide among non-industrialized societies is often more reasonable than purchasing expensive modern pharmaceuticals (Edgar *et al.*, 2002). In traditional system of medicine, different parts of *Phyllanthus reticulatus* Poir. are used for curing various ailments. Bark is used as an astringent and diuretic. Leaves have antidiarrheal properties and roots are used in asthma. Fruits of the plant are used in inflammation (Sharma *et al* 2013). Thin Layer Chromatography (TLC) is a separation technique that is used in the identification of a phytochemical constituent. This technique is used to separate non-volatile mixtures (Harry W *et a*l. 1989). Thin-layer chromatography is completed on a sheet of glass, plastic, or aluminium foil, that is coated with a thin layer of adsorbent material, typically silica gel, cellulose or aluminium oxide. This layer of adsorbent is works as the stationary phase. After applying the sample on the plate, a solvent or solvent mixture (mobile phase) was drawn up the plate through capillary action. Because different analytes ascend the TLC plate at different rates, separation is achieved (Vogel A.I *et al*., 1996). By changing the solvent, or perhaps using a mixture, the separation of components (measured by the R_f value) can be adjusted (Fair *et al.*, 2008).

METHODS AND MATERIALS

Collection and Preparation of plant material

The leaves of *Phyllanthus reticulatus* Poir. were collected from Narayanganj, Bangladesh. The leaves were then washed and sun dried. The plant was authenticated from Bangladesh National Herbarium (Accession no.- 40861). The dried leaves were grinded by a grinder to coarse powder and preserved for extraction purposes.

Extraction of the plant material

The extraction process was maceration. 50gm of plant material was soaked in 250 ml of 90% methanol in water with occasional shaking and stirring for 10 days. The mixture was then filtered (firstly with cotton plug and then with filter paper) and volume of the filtrate was reduced on a water bath. The weight of crude extract was 3gm.

Phytochemical screening

Test for Carbohydrates

2ml of extract was taken in a test tube and 10 ml water was added to it. 2 drops of 25% ethanolic α- naphthol and 2ml concentrated sulphuric acid was added carefully to the mixture. No appearance of reddish brown color at the junction indicated the absence of carbohydrates (Sofowora, 1993).

Test for Alkaloids

Few drops of Dragendorff's reagent was added to 2ml of crude extract. The orange red color precipitation indicated the presence of alkaloids (Sofowora, 1993).

Test for Glycoside

1ml of extract was mixed with 1ml of glacial acetic acid. 5-6 drops of 1% ferric chloride solution was added then. No brown color ring at the top indicate glycoside's absence (Patil and Nasreen, 2016).

Test for Coumarins

3 ml of 10% NaOH was added to 2ml of extract in a test tube. Yellow coloration of the mixture confirmed the presence of coumarins. (Rizk, 1982).

Test for Tannin

A portion of crude extract was dissolved in water and then it was filtered. 1% $FeCl_3$ solution was added to the filtrate. The color of the mixture was changed to bluish black that indicated the presence of tannin (Trease and Evans, 2002).

Test for Terpenoids

2 ml of extract was added to 2 ml of chloroform. Concentrated sulphuric acid 2-3 drops was carefully added to form a layer. A reddish brown coloration of the interface indicates the presence of terpenoids (B.K. Das et al., 2014).

Test for Saponin

0.5gm extract was dissolved in 10 ml distilled water and shaken for 30 sec. Then the mixture was allowed to stand for 30 minute and the appearance of froth was observed (Kumar et al., 2009).

Test for Flavonoids

3-4 drops of 20% NaOH solution was added in 2ml of extract. Formation of intense yellow color which become colorless when 4-5 drops of diluted HCl was added indicates the existence of Flavonoids (Ugochukwu et al., 2013).

Test for Steroid (Salkowski's Test)

2 ml of extract was treated with 2 ml of chloroform and equal amount of concentrated sulphuric acid was added. Reddish ring at the junction indicates the presence of the steroids (Sofowora, 1993).

Thin Layer Chromatography (TLC)

TLC (Thin Layer Chromatography) plate preparation: Glass plates were used to prepare Thin Layer Chromatography plate. A slurry was made by mixing silica gel and distilled water in the proportion of 1:2. The slurry was spread to the glass plate by spreader. The plates covered with silica gel were kept in room temperature for few minutes to make the stable. The plates were then dried by oven dryer at 60°C temperature.

Sample spotting on the plate: Sample was applied on the TLC plate by capillary tube and the kept in different solvent system which have been used as mobile phase. The sample was moved by capillary action through mobile phase and then the plate was taken from the solvent. Then the plate was kept in an iodine tank. After five minutes the plate was observed for the detection of spot. The distance travelled by the solvent and compound were measured with measuring scale.

Determination of the R_f value

The retardation factor, or R_f, is defined as the distance traveled by the compound divided by the distance traveled by the solvent.

$$R_f = \frac{\text{distance traveled by the compound}}{\text{distance traveled by the solvent front}}$$

RESULTS AND DISCUSSION

Table 1: Phytochemical constituents of *Phyllanthus reticulatus* Poir.

SI. No.	Constituent	Result
1.	Alkaloids	+
2.	Glycosides	-
3.	Terpenoids	+
4.	Carbohydrates	-
5.	Saponins	+
6.	Tannins	+
7.	Coumarins	+
8.	Flavonoids	+
9.	Steroids	+

Key: + = Positive, - = Negative

Table 2: Results of separation by Thin Layer Chromatography (TLC).

Solvent	Ratio	Observation
Ethyl acetate: Isopropyl alcohol: acetic acid: water	80: 10: 5: 5	No separation
Ethanol: Methanol: Water	5: 1 :1	No separation
Ethyl acetate: Methanol	15: 1	No separation
Ethyl acetate: Methanol	10: 1	No separation
Ethyl acetate: Methanol	9: 1	No separation
Ethyl acetate: Methanol	50: 50	No separation
Ethyl acetate: Methanol	25: 75	No separation
Ethyl acetate: Methanol	40: 60	No separation
Pet ether: Ethyl acetate	25: 75	No separation
Pet ether: Ethyl acetate	50: 50	No separation
Pet ether: Ethyl acetate	75: 25	Separation occurred, 3 spots were observed

Table 3: R_f value of separated compounds.

Compound	Distance traveled by compound	Distance traveled by solvent	Rf value
A	3.4	8.5	0. 40
B	4.3	8.5	0. 51
C	5.3	8.5	0. 62

The phytochemical screening of *Phyllanthus reticulatus* Poir. state that it contains tannins, saponins, alkaloids, coumarins, terpenoids, steroids and flavonoids.

Eleven solvent system was used to separate the methanolic fraction of the crude extract of *Phyllanthus reticulatus* Poir. by Thin Layer Chromatography. One solvent system (Pet ether: Ethyl acetate = 75: 25) made the separation of three compounds and other ten did not. The R_f value of these compounds were 0.40, 0.51 and 0.62.

CONCLUSION

This study shows the presence of tannins, saponins, alkaloids, coumarins, terpenoids, steroids and flavonoids in the methanolic extract of *Phyllanthus reticulatus* Poir. of Euphorbiaceae family. This study also confirms the separation of different compounds by Thin Layer Chromatography which can be identified by further investigations.

ACKNOWLEDGEMENT

I am expressing my gratitude to Department of Pharmacy, Primeasia University to provide me the facilities to conduct my research work.

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