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Qualitative and Quantitative Phytochemical Screening and Chemical Fingerprint Analysis of Herbal Plant *Phyllanthus niruri* using HPTLC

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Abstract

This study focused to develop the fingerprint profile of *Phyllanthus niruri* through high performance thin layer chromatography. Various solvent extracts such as ethanol and water were prepared and phytochemical screening was made using standard procedures. The preliminary phytochemical analysis of two extracts of the plant showed the presence of secondary metabolites *viz.,* alkaloids, triterpenoids, proteins, amino acids, flavonoids and steroids. The total flavonoid contents of leaves were significantly higher than those revealed in stem and root. The total phenolic contents of *Phyllanthus niruri* leaves (aqueous extract), leaves (ethanol extract) and root (ethanol extract) were 5.71 mg GAE/100 mg extract, 7.66 mg GAE/100 mg and 5.63 mg GAE/100 mg extract respectively. Methanol extract of *Phyllanthus niruri* was subjected to HPTLC analysis due to it is most effective, highly polar universal solvent for extraction of phytoconstituents. Among the samples, leaves extract resolved maximum number of spots followed by stem extract and root extract. It can be concluded that HPTLC fingerprinting of *Phyllanthus niruri* may be useful in differentiating the species from the adulterant and authentication of this herbal plant in the medical field and systematic plant studies.

Keywords: High performance thin layer chromatography (HPTLC); Phytochemical screening; Total flavonoid contents (TFC); Total phenolic contents (TPC); *Phyllanthus niruri*.

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1. Introduction

Plants are backbone of life, and generate a number of chemical compounds that have biological activity and in ancient times, plants were the only treatment option for humans and they played excellent role in therapeutic field [1]. The bioactive constituents (phytochemical constituents) mostly present in various parts of plant like leaves, roots, stem and bark that have defence system and provide protection from various diseases such

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as alkaloids, terpenoids, flavonoids, sugar, proteins, saponins, phenols, tannins and quinones. Medicinal plants have pharmacological activity due to presence of these phytochemicals. More than 4,000 phytochemicals have been discovered in this respect [2]. These phytochemicals are estimated by a variety of techniques such as spectroscopy and chromatography. Chromatography can easily detect the presence and concentration of therapeutically important chemical components in herbal preparation [3,4]. HPTLC is the effective and powerful tool for analysis of fingerprinting profile of plant extract since long and for estimation of chemical, biomedical analysis and check adulterations [5-8].

There are more than three hundred genera with about five thousand species in the Euphorbiaceae around the world. In Phyllanthus genus, there are about 750 species of the family Euphorbiaceae [9]. Phyllanthus signifies "leaf and flower" because the flower and fruit, appears to become one with the leaves. *Phyllanthus niruri* (syn *P. amarus, P. fraternus* Webster, family - Euphorbiaceae) is very much related to other species of Phyllanthus [10]. *Phyllanthus niruri* originates in India, and is usually found as a winter weed. Chhattisgarh state has medicinal tradition of this plant. It is utilized for treatment of snake bites in India. [11,12]. It was first identified in Central and Southern India in eighteenth century [13]. It is indigenous to the tropical areas throughout the world, including, Southern India, Bahamas, Ghana, China, Nigeria and also rainforests of the Amazon [14]. One of the identification characteristics of *Phyllanthus niruri* is that it has phyllanthid branches with the presence of fruit and flowers under the leaves [15]. Different parts of plant like root, fruits, milky juice, leaves and whole plants are used as medication. It is used in all the medicinal system such as Ayurvedic, Chinese and Unani traditional medicine. The various ayurvedic formulations contain *Phyllanthus niruri* extract [16]. In India, it is a significant component of liver tonics including Liv.-52 [17]. In Brazil, *Phyllanthus niruri* are used medicinally to alleviate kidney stone and intestine infection [18,19]. It holds a reputed position in medicinal systems even though, problematic weed for farmer. Recently, it has attracted the attention of researchers, because of its hepatoprotective properties and also shown clinical efficacy in viral Hepatitis B [20]. In the present study, the qualitative and quantitative phytochemical screening and HPTLC finger printing of methanol extracts of leaves, stem and root of *Phyllanthus niruri* has been performed to identify the phytochemical constituents and generate fingerprinting profile for authentication and further studies of this plant.

2. Material and Methods

2.1. *Collection and identification of plant material*

Fresh and healthy plant parts of *Phyllanthus niruri* (leaves, stem and root) were collected in a separate sterile bag during the month of July to October from different locations of Chitrakoot, Satna District, Madhya Pradesh, and identified by using morphometric investigation.

2.2. *Extraction procedure of plant sample*

The collected samples were thoroughly washed in tap water and rinsed with distilled water and slice into small pieces and shade dried for 3 to 4 weeks then ground into fine powder using electric grinder. Two different solvents (ethanol and aqueous) were used for extraction of all the defatted powdered plant samples Maceration extraction is commonly used method for small research level. The extraction was done at room temperature. After extraction, different plant sample extracts were filtered using Whatman No.1 paper to obtain plant extracts. Then, filtrates were evaporated by distilling the solvents at low temperature using water bath. Finally, all the plant extracts stored at 4 °C in labelled sterile bottles until further use.

2.3. *Methods of phytochemical screening*

The methods of Khandelwal, Kokate, and Tiwari were adopted for the qualitative screening of phytochemicals in selected plant sample [21-23].

2.4. *Determination of total phenolic content*

The total phenolic content (TPC) of *Phyllanthus niruri* extracts were determined by the modified Folin-Ciocalteu method [24]. Firstly, 2 mL of solution of selected plant extracts or standard were taken in 10 mL volumetric flask separately. 1 mL of Folin-Ciocalteu reagent (diluted with distilled water 1:10 (v/v)) and one ml of sodium carbonate was added to each flask. Subsequently, reaction mixture was vortexed for 15 sec and left for 15 min at room temperature for color development. After incubation of reaction mixture, absorbance was recorded at 765 nm by spectrophotometer. The standard curve produced with varying concentrations of Gallic acid (R_2 = 0.9986) was used to determine the amount of phenolic content in plant sample. The TPC was expressed as percentage mg Gallic acid equivalents /100 mg dry weight of plant sample.

2.5. *Total flavonoid content estimation*

Aluminium chloride method was adopted to determine total flavonoid content of different parts of *Phyllanthus niruri* [24]. For estimation of total flavonoid contents (TFC), 1 mL of (2 %) $AlCl_3$ methanolic solution was added into 3 mL of different dilution of standard solution of Quercetin (5, 10, 15, 20 and 25 µg/mL) or plant extract and left for 15 min at room temperature. Subsequently, the solution was mixed and the absorbance was recorded against a freshly prepared blank reagent at 420 nm using spectrophotometer. The standard curve produced with varying concentrations of Quercetin (R_2 = 0.999) was used to determine total flavonoid content of plant sample. TFC of the plant extract was presented as percentage of Quercetin equivalent per mg/100 mg dry weight of the sample.

2.6. *HPTLC finger printing analysis*

Extraction was made using 50 mg sample of leaves, stem and root of *Phyllanthus niruri* using methanol solvent. Subsequently filtered sample solution was applied on precoated silica gel aluminium plate F254 (0.2 mm layer thickness) Merck, Mumbai, using Camag linomat-5 sample applicated Camag syringe. Different suitable mobile phases consisting of toluene: ethyl acetate (7:3) for leaves, toluene: ethyl acetate: diethyl ether (5:3:2) for stem, toluene: ethyl acetate: diethyl amine (7:2:1) for root and toluene: ethyl acetate: diethyl amine (7:3:0.5) for leaves, stem and root of *Phyllanthus niruri* were used for development of Plates. Linear ascending development was carried out in 10×10 cm twin trough glass chamber with SS/LID equilibrated with mobile phase. The length of chromatogram run was 9 cm. After development, TLC plates were removed and dried on pre heated hot air oven at 105 °C for 5 to 10 min and visualization of spots was made before and after derivatization (with 5 % methanolic H_2SO_4 reagent) at 254 nm, 366 nm and under UV light. Photo documentation system (Camag- Reprostar 3140604 with software) was used for documentation of all the images. The spots observed were detected and their Rf values recorded [25-26].

3. Results

3.1. *Percentage Yield of Phyllanthus niruri*

The *Phyllanthus niruri* leaves extract exhibited higher yield 5.8 % followed by stem extract 4.6 % and root extract 2.7 % with aqueous solvent respectively. The leaves extract of *Phyllathus niruri* shows higher percentage yield 5.2 % followed by stem extract 4.8 % and root extract 2.8 % respectively with ethanol solvents (Fig. 1).

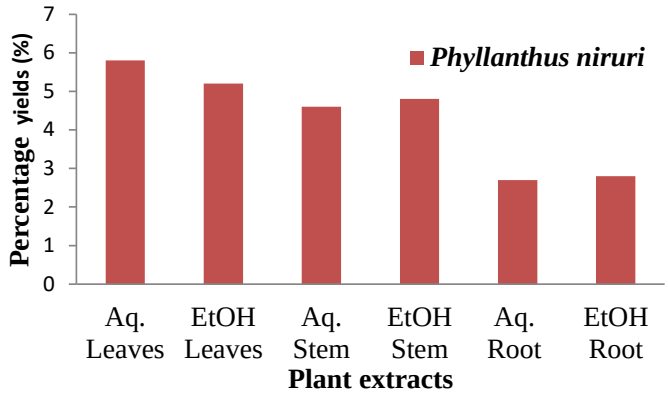

Fig. 1. Percentage yield of plant extracts. Aq- aqueous, EtOH- ethanol.

3.2. *Phytochemicals screening of Phyllanthus niruri with aqueous solvent*

Phytochemical analysis for aqueous extract of leaves, stem and root of *Phyllanthus niruri* is depicted in Table 1. All parts (leaves, stem and root) showed positive test for flavonoids, amino acids, carbohydrates and proteins. Steroid and alkaloids were absent in leaves, stem and root, but diterpenes was present in only root extract. Saponins showed positive test with *Phyllanthus niruri* (stem and root).

3.3. *Phytochemicals screening of Phyllanthus niruri with ethanol solvent*

Phytochemical analysis for ethanol extracts of leaves, stem and root of *Phyllanthus niruri* are tabulated in Table 1. Flavonoids and phenols are present in ethanol extract of *Phyllanthus niruri* leaves and root. Amino acids and proteins showed positive test with leaves and stem extract. Saponins were also present in stem and root extracts of the plant. The phytochemical analysis of ethanol extract of leaves, stem and root of *Phyllanthus niruri* showed negative results for alkaloids, glycosides, steroids and carbohydrates.

Table 1. Results of Phytochemical Screening of *Phyllanthus niruri*

S. No.	Constituents	Aqueous			Ethanolic		
		Leaves	Stem	Root	Leaves	Stem	Root
1	Alkaloids	-	-	-	-	-	-
2	Glycosides	+	-	-	-	-	-
3	Flavonoids	+	+	+	+	-	+
4	Steroid	-	_	-	-	-	-
5	Phenolics	+	-	-	+	-	+
6	Amino Acids	+	+	+	+	+	-
7	Carbohydrate	+	+	+	-	-	-
8	Proteins	+	+	+	+	+	-
9	Saponins	-	+	+	-	+	+
10	Diterpenes	_	_	+	-	+	-

+: Indicates the presence of phytochemicals, -: Indicates the absence of phytochemicals

3.4. *TFC of Phyllathus niruri*

Quantification of TFC of the plant revealed variation in concentration of flavonoid in plant sample. The TFC of *Phyllanthus niruri* (aqueous and ethanolic extract) are summarized in Fig. 2. The TFC of leaves were significantly higher than those revealed in stem and root. The ethanolic extract of the plant leaves had comparatively higher flavonoid content.

3.5. *TPC of Phyllathus niruri*

Fig. 2 reveals the results of TPC in the aqueous and ethanol extracts of leaves, stem and root. The results showed that aqueous extract of leaves contain less TPC than ethanol extract. The plant extract showed the highest concentration of flavonoids followed by phenolic content in leaves when compared with stem and root thus the leaves extract of plant may hold better therapeutic application.

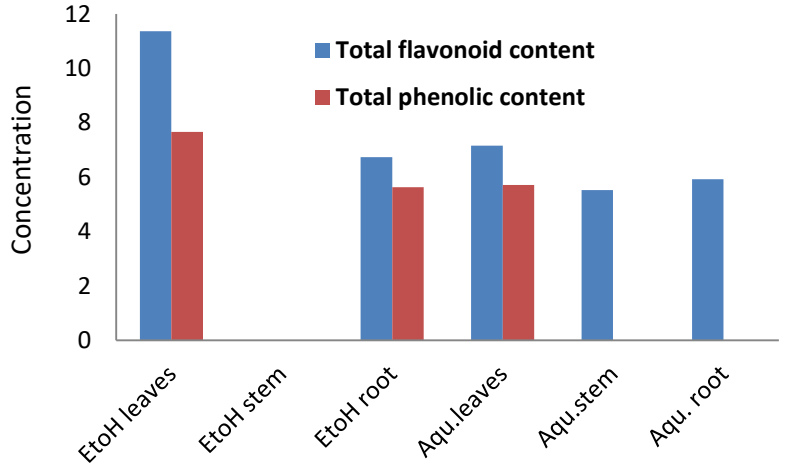

Fig. 2. Total flavonoid contents and phenolic contents of *Phyllanthus niruri*. Aq- aqueous, EtOH- ethanol.

3.6. *Comparative study of Chromatographic fingerprinting analysis of leaves, stem and root of Phyllanthus niruri*

The HPTLC chromatogram and Rf values for methanol extracts of leaves, stem and root of *Phyllanthus niruri* after scanning at 254 nm, 366 nm before derivatization and 254 nm, 366 nm and under ultraviolet light after derivatization are depicted in Table 2 and Fig. 3.

Distinct chromatograms were obtained for the methanol extract of the leaves, stem and root parts of *Phyllanthus niruri*. Among the plant samples of *Phyllanthus niruri*, leaves extract resolved maximum number of spots by 9, 10, 10, 13, 14 and 12 at 254 nm, 366 nm and under ultraviolet before derivatization and at 254 nm, 366 nm and under ultraviolet after derivatization respectively. It was followed by stem extract of *Phyllanthus niruri* which had 2, 10, 6, 12, 14 and 4 at 254 nm, 366 nm and under ultraviolet before derivatization and at 254 nm, 366 nm and under ultraviolet after derivatization respectively. The HPTLC analysis of root extract exhibited a number of spots by 10, 2, 8 and 3 at 366 nm before derivatization and 254 nm, 366 nm and under ultraviolet after derivatization.

Table 3. Showing comparative R_f Values of HPTLC Finger prints profile of leaves, stem and root of *Phyllanthus niruri.*

R_f Values	At 254 nm before derivatization		
	Leaves	Stem	Root
R_f 1	0.05(light black)	0.90(light black)	Not appear
R_f 2	0.23(light black)	0.97(light black)	-
R_f 3	0.34(light black)	-	-
R_f 4	0.41(light black)	-	-
R_f 5	0.48(light black)	-	-
R_f 6	0.59(black)	-	-
R_f 7	0.69(light black)	-	-
R_f 8	0.90(black)	-	-
R_f 9	0.97(black)	-	-
R_f Values	At 366 nm before derivatization		
	Leaves	Stem	Root
R_f 1	0.05(red)	0.04(red)	0.04(light red)
R_f 2	0.07(red)	0.07(light red)	0.17(light red)
R_f 3	0.49(red)	0.26(red)	0.27(light red)
R_f 4	0.57(light red)	0.31(red)	0.32(light red)
R_f 5	0.70(red)	0.40(light red)	0.40(light red)
R_f 6	0.76(light red)	0.48(light red)	0.47(light red)
R_f 7	0.82(light red)	0.69(red)	0.69(light red)
R_f 8	0.85(light red)	0.83(red)	0.83(red)
R_f 9	0.90(red)	0.91(red)	0.87(blue)
R_f 10	0.96(red)	0.96(light red)	0.90(red)
R_f Values	Under Ultra Voilet before derivatization		
	Leaves	Stem	Root
R_f 1	0.05(light black)	0.04(light black)	NA (Not appear)
R_f 2	0.06 (light yellowish green)	0.26(light green)	-
R_f 3	0.22 (green)	0.33(light yellow)	-
R_f 4	0.30 (green)	0.47(light green)	-
R_f 5	0.34(green)	0.90(light black)	-
R_f 6	0.42(light green)	0.98(yellow)	-
R_f 7	0.47(light green)	-	-
R_f 8	0.69 (blue)	-	-
R_f 9	0.90(light blue)	-	-
R_f 10	0.97(yellowish pink)	-	-
R_f Values	At 254 nm after derivatization		
	Leaves	Stem	Root
R_f 1	0.06(Red)	0.05(red)	0.60(whitish)
R_f 2	0.07(brown)	0.06(grey)	0.90(light red)
R_f 3	0.08 (brown)	0.07(blue)	-
R_f 4	0.10(light red)	0.15(light red)	-
R_f 5	0.42(brick red)	0.27(red)	-
R_f 6	0.48(light red)	0.33(light red)	-
R_f 7	0.54(blue)	0.48(light red)	-
R_f 8	0.60(blue)	0.60(whitish)	-
R_f 9	0.62(whitish)	0.69(light red)	-
R_f 10	0.70(red)	0.82(whitish red)	-
R_f 11	0.85(whitish yellow)	0.91(red)	-
R_f 12	0.91(red)	0.98(brown)	-

R_f 13	0.97(black)	-	-
R_f Values	At 366 nm after derivatization		
	Leaves	Stem	Root
R_f 1	0.05(light red)	0.05(red)	0.05(whitish green)
R_f 2	0.06(white)	0.06(sky blue)	0.27(light red)
R_f 3	0.07 (red)	0.07(light pink)	0.39(light red)
R_f 4	0.42 (red)	0.15(light red)	0.59(whitish)
R_f 5	0.48(red)	0.27(light red)	0.83(light red)
R_f 6	0.54(blue)	0.32(light red)	0.86(blue)
R_f 7	0.57(dark red)	0.41(light red)	0.90(red)
R_f 8	0.61(white)	0.48(light red)	0.97(light brown)
R_f 9	0.70(red)	0.59(whitish)	-
R_f 10	0.76(light red)	0.69(red)	-
R_f 11	0.83(light red)	0.83(pink)	-
R_f 12	0.90(red)	0.86(light blue)	-
R_f 13	0.97(black)	0.90(red)	-
R_f 14	-	0.97(brown)	-
R_f Values	Under ultra violet after derivatization		
	Leaves	Stem	Root
R_f 1	0.06(light black)	0.06(light black)	0.05(light black)
R_f 2	0.24(green)	0.58(light brown)	0.59(light brown)
R_f 3	0.30(green)	0.90(light blue)	0.97(light brown)
R_f 4	0.34(green)	0.97(brown)	-
R_f 5	0.42(brown)	-	-
R_f 6	0.49(brown)	--	-
R_f 7	0.55(light black)	-	-
R_f 8	0.60(brown)	-	-
R_f 9	0.69(light green)	-	-
R_f 10	0.85(brown)	-	-
R_f 11	0.89(blue)	-	-

4. Discussion

The results of percentage yield showed that all the solvents used for extraction were able to extract phytochemicals present in different plant parts but with varied quantities. Phytochemical screening of ethanolic extracts of leaves revealed the presence of glycosides, flavonoids, phenols, amino acids, proteins and carbohydrates in *Phyllanthus niruri* which is in agreement with the results of Ajibua *et al*. [27], who determined alkaloids, saponins flavonoids, tannins and phenols in *Phyllanthus niruri*. The phytochemical screening results of *Phyllanthus niruri* agreed with the results of Samali *et al*. [28], who determined the presence of alkaloid, sterols, carbohydrates, flavonoids, tannins and resins. The total flavonoid content was relatively more abundant in *Phyllanthus niruri* ethanolic leaves extract than other extracts. Earlier works have also shown total Phenol and total flavonoid of *Phyllanthus niruri* [29]. Furthermore, there are less study reported on the phytochemical screening and chromatography fingerprinting profile of *Phyllanthus niruri* leaves, stem and root (aqueous and ethanolic extracts) in the literature.

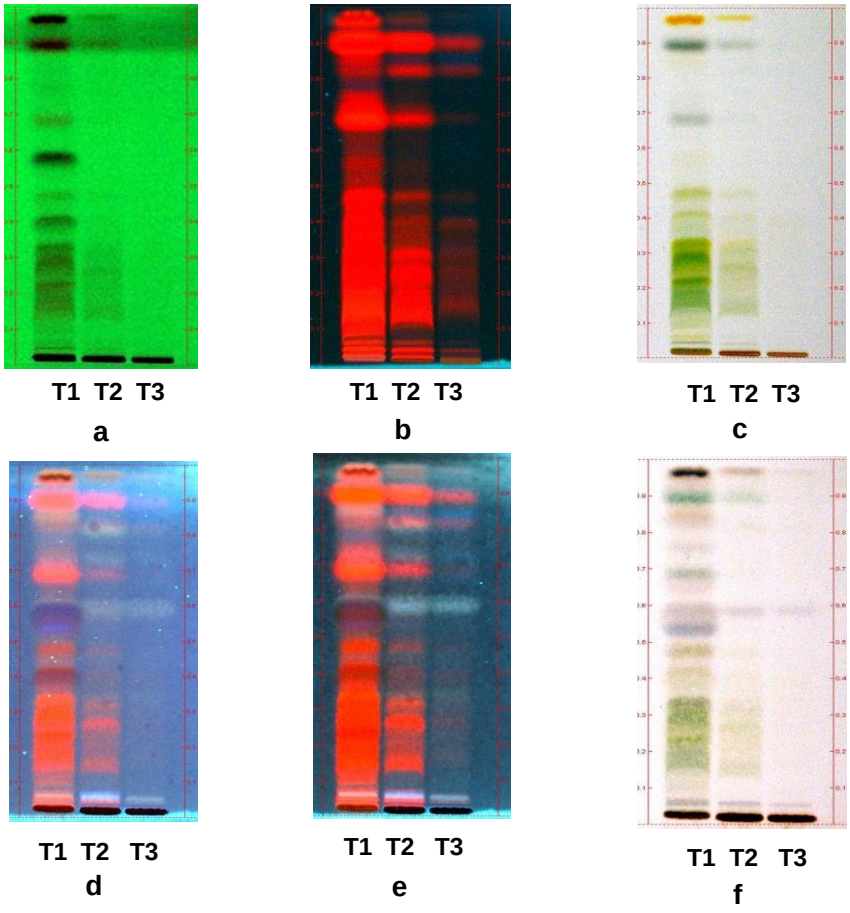

Fig. 3. Comparative HPTLC fingerprinting analysis of *Phyllanthus niruri* leaves, Stem and Root. Frame a, b, c, d, e and f shows different spots at 254 nm, 366 nm and under ultraviolet before derivatization and at 254 nm, 366 nm and under ultraviolet after derivatization respectively. Tracks T1 = Test solution of leaves, T2 = Test solution of stem, T2 = Test solution of root.

5. Conclusion

The present study suggested that *Phyllanthus niruri* leaves contained many phytoconstituents, validated their traditional use in treatment of various ailments. This is the first report which evaluates the fingerprinting profile of different parts of this plant. The HPTLC fingerprinting of leaves, stem and root extracts of *Phyllanthus niruri* may be useful in correct identification of this plant and it is useful in differentiating the species from the adulterant and used for authentication of this herbal plant in the medical field for production of therapeutic products and also in systematic plant studies.

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