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Qualitative and Quantitative Study of Phytochemical Analysis of Medicinal Plant *Phyllanthus niruri* L and Evaluation of its Bioactive Compounds

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Abstract

Phyllanthus niruri L is one of the most valuable medicinal plants both traditionally and scientifically. The phytochemical analysis reveals the presence of potent bioactive compounds that can be effectively used for the preparation of better herbal drugs. The aim of this study to determining the potent bioactive compounds present in the leaf extract of *phyllanthus niruri*. L by qualitative and quantitative analysis with standard procedure. Qualitative analysis of Tannins, Saponins, Flavonoids, Alkaloids, Proteins, Steroids, Anthraquinones, Phenols, Terpenoids and Carbohydrates in *Phyllanthus niruri* of five different extracts Methanol, Acetone, Chloroform, Aqueous and Ethyl acetate were analyzed. Tannins showed highly positive (+++) in aqueous extract, moderately present in methanol extract (++) and absent in acetone, chloroform and ethyl acetate extracts. The saponin showed highly positive in aqueous, moderately present in chloroform and acetone extracts but absent in methanol and ethyl acetate extracts, the flavonoid showed highly positive in aqueous, moderately present in chloroform, methanol and ethyl acetate extracts but it was absent in acetone extracts. The alkaloid showed highly positive in aqueous, methanol and ethyl acetate extracts and moderately present in chloroform and acetone extracts. The protein showed highly positive in aqueous and methanol extracts and positive in chloroform, acetone and ethyl acetate extracts. The steroids showed highly positive in methanol extracts moderately positive (++) in aqueous and it was absent in chloroform, acetone and ethyl acetate extracts. The anthraquinonen showed highly positive in aqueous and positive (+) in methanol and it was absent in chloroform, acetone and ethyl acetate extracts. The phenols showed highly positive in aqueous and methanol extracts, positive in chloroform extract and it was absent in acetone and ethyl acetate extracts. The Terpenoids showed highly positive in aqueous, chloroform and methanol extracts and it was absent in acetone. The carbohydrates showed highly positive (+++) in aqueous, chloroform, methanol and ethyl acetate extracts and positive in acetone. Among the extracts the aqueous extracts showed presence of more number of phytochemical compounds. Secondly the methanol showed more number of phytochemicals. The remaining extracts chloroform, ethyl acetate and acetone showed less number or even absence of phyto chemical compounds. The quantitative estimation of alkaloids flavonoids and phenolic compounds in methanol and aqueous extract of *Phyllanthus niruri* havealso been carried out.The Aqueous extract showed more amount of Alkaloids (0.08 µg/ml), Flavonoids (275 mg/ml) and Phenolic (158 mg/ml) compounds.

***Key words*:** *Phyllanthus niruri*, Phytochemical analysis, Tannins, Saponins, Flavonoids, Alkaloids, Phenols, Terpenoids

Plants have been used in traditional medicine since a long time. About 13,000 plant species have been used as drugs throughout the world, and approximately 25% of the current materiamedica are derived from plants in the form of teas, extracts, or pure substances [1-2]. Traditional medicine using plant extracts continues to provide health coverage for over 80% of the world's population, especially in the developing countries in the world [3]. In the Democratic Republic of Congo (DRC) among the species used in the treatment against malaria, *Phyllanthus niruri* is well positioned for different previous studies on this plant [4-6]. *Phyllanthus niruri* is one of the most important medicinal plants used in different region in the world for the treatment of various disease such as jaundice, asthma, hepatitis, flu, dropsy, diabetes, fever causing by malaria [7-9]. But its availability is drastically decreasing because of numerous harvests. *Phyllanthus* is a large genus of shrubs, trees and rare herbs of the family Euphobiaceae, comprising more than 600 species, of which *Phyllanthus accuminatus*, *Phyllanthus amarus*, *Phyllanthus pulcher*, *Phyllanthusniruroides*, *Phyllanthus anisolobus*, *Phyllanthusorbiculatus*, *Phyllanthus emblica*, *Phyllanthuso xyphyllus*, *Phyllanthus flexuosus*, *Phyllanthusraticulatus*, *Phyllanthus fraternes*, *Phyllanthus simplex*, *Phyllanthus mullernus*, *Phyllanthus urinaria*, *Phyllanthus mytrifolis*, *Phyllanthusvirgatus*, *Phyllanthus niruri* and *phyllanthus watsonii* were investigated for their phytochemical and

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pharmacological properties. The genus is found almost over all warmer parts of the world [10]. Among the Phyllanthus species, *Phyllanthus niruri* is a small erect annual herb growing up to 30-40 cm in height and is indigenous to the Amazon rainforest and other tropical areas, including South East Asia, Southern India and China [11]. Its leaves are 7-12 cm long and they are alternate, sessile oblong. It has small off-white-greenish flowers, which are solitary, auxiliary, pedicellate, a petalous and monoecious. *Phyllanthus amarus* and *Phyllanthus sellowianus* are closely related to phyllanthusniruri in appearance. Phytochemical contents and history, but they are found in drier regions of India and Brazil, and even in Florida and Texas. In a recent report, cladistic analysis indicated that the *phyllanthus* genus is paraphyletic and therefore the two problematic and confusing species, *Phyllanthus niruri* and *Phyllanthus amarus*, are two individual species [12]. *Phyllanthus niruri* has a long history in herbal medicine system such as Indian Ayurveda, Traditional Chinese Medicine and Indonesian Jamu. The whole plant is used as remedies for many conditions such as dysentery, influenza, vaginitis, tumours, diabetes, diuretics, jaundice, kidney stones and dyspepsia. The plant is also useful for treating hepatotoxicity, hepatitis B, hyperglycemia and viral and bacterial disease [13]. *Phyllanthus niruri* has been used in Ayurvedic medicine for over 2000 years and has a wide number of traditional uses for jaundice, gonorrhea, frequent menstruation and diabetes. It is an important medicinal plant in Jamu, a well –known Indonesian traditional herbal medicine to treat various disease. In Jamu preparations, the plant is used as antiviral and hepatoprotective agent. In Malaysia, *phyllanthus niruri*, known as Dukonganak, is used internally for diarrhea, kidney disorders, gonorrhea and coughs [10].

Phyllanthus niruri is called Chanca Piedra in Spanish, which means stone breaker, because it has been used as an effective remedy to eliminate gallstones, kidney stones and other kidney disorders. In a preclinical study, *Phyllanthus niruri* aqueous extract exhibited a potent and effective non-concentration-dependent inhibitory effect on calcium oxalate (CaOx) crystal formation [14]. This response was present even at higher concentrations. This may explain why it has long been used in traditional medicine as a preventive to kidney stone formation [15]. Brazillian herbal medicine, it is called Quebra Pedra and is considered an excellent remedy for hydropsy, urinary and bladder infections. It is also used to cure kidney disorders, hepatitis and diabetes [16-18]. In India, where it is called pitirishi or Budhatri, it is a common household remedy for asthma, bronchitis, coughs, extreme thirst, anaemia, jaundice and tuberculosis [19]. *Phyllanthus niruri* has been the subject of much research to investigate the active constituents and their pharmacological activity, beginning in the mid-1960s. Ottow was the first to work on *Phyllanthus niruri* and reported the isolation of *Phyllanthin* in 1891 [20]. It has a rich source of phytochemicals, many of which have been found only in *Phyllanthus niruri* [19].

Many of the active constituents to which the biological activity of *Phyllanthus niruri* has been attributed include lignans, stannins, coumarins, terpenes, flavonoids, alkaloids, saponins and phenylpropanoids, which have been found in the leaves, stem and roots of this plant. Common lipids, sterols and flavonols also occur in the plant [19]. Indian and Brazilian research groups were the first to conduct studies relating to the medicinal properties of *Phyllanthus niruri* since this plant is indigenous to their areas with a long history of use by their inhabitants [21]. Brazilian researchers documented the antispasmodic activity of an alkaloid of *Phyllanthus niruri* [22]. *Phyllanthus niruri* gained worldwide attention in the late 1980s due to its activity against hepatitis B [23]. An alkaloid extract of *Phyllanthus niruri* demonstrated smooth muscle relaxation effect specific to the urinary and biliary tracts [24]. The anti-hepatotoxic activity of *Phyllanthus niruri* has been attributed to two novel lignans, phyllanthinand hypophyllanthin [25]. Glycosides (quercitrin and geraniin) found in *Phyllanthus niruri* demonstrated aldose reductase inhibitory (ARI) activity in studies conducted by a Japanese research group in 1988 and 1989 [26-27]. The ARI effect was also due to the presence of another ellagitannin phytochemical, ellagic acid [27]. The plant also possessed potent analgesic activity against pain models in rats [28-29]. The diuretic, hypotensive and hypoglycaemic effects of *Phyllanthus niruri* were documented in a human study, which showed a significant diuretic effect [30]. Similar studies in man revealed that *Phyllanthus niruri* caused reduction in the systolic blood pressure in non-diabetic hypertensive patients and reduction of blood glucose in diabetic patients [31-32]. In-vitro and In-vivo studies showed that extracts of *Phyllanthus niruri* effectively protected against liver damage induced by various chemical liver toxins [33-34]. Indian researchers reported that *Phyllanthus niruri* was an effective single drug in the treatment of jaundice in children [35]. In recent studies, the protein fraction of *Phyllanthus niruri* demonstrated protection of liver tissues against oxidative stress in mice [36-37]. So, an attempt is made in this study to screen the phyto chemical compounds present in *Phyllanthus niruri* of different extracts.

MATERIALS AND METHODS

Sample preparation

Phyllanthus niruri L leaves were collected from the local market of cheyyar taluk, Thiruvannamalai district of Tamil Nadu. The leaves were washed with water to remove the dust particles. The washed leaves were dried at room temperature and were powdered mechanically. The powdered leaves were extracted with the help of Soxhlet apparatus with methanol, Acetone, Chloroform, Aqueous and Ethyl acetate as a solvent at 75°c for 6 hours. The extracts were dried and refrigerated at 4 °C for further usage.

Phytochemical screening

The phytochemical screenings were performed by the following standard procedures mentioned by [38]. Screenings for the presence of Tannins, Saponins, Flavonoids, Alkaloids, Protein, Steroids, Anthraquinones, Phenols, Terpenoids and Carbohydrates.

Phytochemical analysis [38]

1. Test for tannins

1 ml of sample was taken, to that few drops of 0.1% ferric chloride was added and observed for brownish green or blue-black coloration confirms the presence Tannins.

2. *Test for saponins*

1 ml of sample was taken, to that 2 ml of water was added. The suspension was shaken in a graduated cylinder for 15 minutes. A layer of foam indicates the presence of saponins.

3. *Test for flavonoids*

1 ml of sample was taken, to that add NAOH to observe yellow colour and concentrated hydrochloric acid was added and observed white colour for the presence of flavonoids.

4. *Test for alkaloids*

1 ml of sample was taken, to that few drops of drangandoff reagent was added. A prominent yellow precipitate indicates the test as positive for the presence of alkaloids.

5. *Test for protein*

1 ml of sample was taken, to that few drops of Millon's reagent was added. A white precipitate indicates the presence of protein.

6. *Test for steroids*

1 ml of sample was taken, to those two drops of 10% concentrated sulphuric acid was added and observed for brown colour indicate the presence of steroids.

7. *Test for anthraquinones*

1 ml of sample was taken, to that aqueous ammonia was added and observed for change in colour. Pink, red or violet colour in aqueous layer indicates the presence of anthraquinones.

8. *Test for phenols*

1 ml of sample was taken; to that 3ml of 10% lead acetate solution was added. A bulk white precipitate formed at the surface indicates the presence of phenolic compounds.

9. *Test for terpenoids*

2 ml of chloroform was taken, followed by 3 ml of concentrated sulphuric acid was added to 0.5 ml of the extract. Formation of red brown colour at the interface confirms the presence of terpenoids.

10. *Test for carbohydrates*

0.5 ml of sample was taken, to that 0.5 ml of Benedicts reagent was added and mixed well and then it was placed in the water bath for 2 mins. Formation of colored precipitate indicates the presence of sugars.

Quantitative phytochemical analysis

Estimation of total phenolic content [39]

The total phenolic content (TPC) of the Sample was determined by spectrophotometric method 1 ml of sample (1 mg ml^{-1}) was mixed with 1 ml of Folin-Ciocalteu's phenol reagent. After 5 minutes, 10 ml of 7% NA_2CO_3 solution was added to the mixture, followed by the addition of 13 ml of deionized distilled water and mixed thoroughly. The mixture was kept in dark for 90 min at 23°C, after which absorbance was read at 750 nm. The TPC was determined from extrapolation of calibration curve which was made by preparing gallic acid solution. The TPC was expressed as milligrams of gallic acid equivalents (GAE) per gm of sample.

Total flavonoids estimation

Procedure (*Aluminium Chloride Colorimetric Assay*) [40]

One ml of sample was taken in a Tube, to this, 0.3 ml of 5% sodium nitrite was added, after 5 mins 0.3 ml of 10% aluminum chloride was mixed, 2 ml of 1 M sodium hydroxide was added to neutralize the reaction. A standard curve was prepared with Quercetin solution (200, 400, 600, 800 and 1000µg/ml) as the procedure described earlier. The absorbance readings were recorded for test and standard solutions against blank at 510 nm in UV/Visible spectrophotometer. The total flavonoid content was expressed as µg of QE/g of extract.

Quantitative estimation of alkaloids [41]

1 mg of sample was taken; to this add 5 ml of phosphate buffer (pH 4.7) and 5 ml Bromocresol green (BCG) solution was added. The mixture was shaken well with 4 ml of chloroform. The extracts were collected in 10-ml volumetric flask and then diluted to adjust volume with chloroform. The absorbance of the complex in chloroform was measured at 470nm against blank prepared as above but without extract. Atropine is used as a standard material and compared the assay with atropine equivalent.

RESULTS AND DISCUSSION

The phytochemical analysis of *Phyllanthus niruri* leaf extract using methanol, acetone, chloroform, aqueous and ethyl acetate were given in (Table 1). From the observation of phytochemical analysis, the Tannins showed highly positive in aqueous extract positive in methanol and absent in acetone, chloroform and ethyl acetate. Saponins showed highly positive in aqueous extract, positive (++) in acetone and chloroform and absence of saponins in Methanol and ethyl acetate. Flavonoid showed highly positive in aqueous and positive in methanol, chloroform and ethyl acetate but negative in acetone. The alkaloids showed highly positive in aqueous, methanol and ethyl acetate, positive in acetone and chloroform extracts. Protein showed highly positive in aqueous and methanol but positive in acetone, chloroform and ethyl acetate. The steroids showed highly positive in methanol but positive in aqueous and absent in acetone, chloroform and ethyl acetate extracts. The phenols showed highly positive in aqueous and methanol, low positive in chloroform and negative in acetone and ethyl acetate. Terpenoides showed highly positive in methanol, chloroform and aqueous, positive in ethyl acetate and negative in chloroform. Lastly the carbohydrate showed highly positive in methanol, chloroform, aqueous and ethyl acetate but positive in acetone extract. Among the solvents the aqueous showed more number of phytochemicals with highly positive condition. Acetone showed less number of phytochemicals with negative condition. Among the phytoconstituents carbohydrate showed highly positive in all the solvents except acetone (Positive). The anthraquinones showed negative in acetone, chloroform and ethyl acetate. The results are in agreement with those of Campus and Schor [14] they found more number of phytochemicals constituents in aqueous extracts of *Phyllanthus niruri.* Quantitative estimation of some phytochemicals like alkaloids, flavonoids and phenolic compounds were carried out in two solvents (Methanol and Aqueous) in the present study the result is given in (Table 2). The quantitative estimation study showed that 1 mg of sample powder (*Phyllanthus niruri*) contains 0.069 µg/ml of Alkaloid in methanol extract and 0.080 µg/ml of alkaloid in aqueous extract of *Phyllanthus niruri*. So aqueous extract showed more amount of alkaloid content when compared to methanol.

The quantitative analyses of *Phyllantus niruri* (aqueous and methonolic leaf extracts) of flavonoids, phenolic compound and alkaloids were given in the (Table 2-7; Graph 1-3). The flavonoid content showed 258 mg/ml in methanol extract and 275 mg/ml in aqueous extract. So aqueous extract showed more amount of flavonoids when compared to methanol extract. The Quantitative estimation of phenolic content showed 155 mg/ml in methanol extract and 158 mg/ml in aqueous extract. The phenolic content also showed more amount of phenols present in the aqueous extract when compared to methanol extract. In the present study the aqueous extracts showed more amount of flavonoids, alkaloids and phenolic by quantitative estimation. The results are in agreement with those Row *et al.* [20] thy found out more amount of *Phyllanthus* extract. herbal medicine has been used for centuries for the treatment of various diseases. It is an important part of Ayurveda, Siddha and Yunani

medicine. Different parts of various plants are used by indigenous people across the world to cure wounds, snake bites, abdominal pain, skin infections and several other diseases. In a study by word health organization (WHO), it was estimated that 40% of the world population still depend on herbs and plants as medicine [42]. Several phytochemicals such as vincristine, artemisin, quinine, digoxin have been isolated from plants which have shown a broad range of pharmacological activities [43]. The modern sedentary life style, stress, pollution, junk food and alcohol have exacerbated the harms caused by free radicals. The free radicals are associated with diseases such as diabetes, arthritis, cancer, Parkinson's disease and Alzheimer's disease [44]. The phytochemicals like phenol, flavonoids, saponin, alkaloid and terpenoids can scavenge free radicals. These compounds have previously shown strong, anticancer, antidiabetic, anti-inflammatory and antimicrobial activity [45]. In the present study among the five different solvent extracts the aqueous extract showed more activity of phytochemicals that is highly positive in all the phytoconstituents like tannins, saponins alkaloids, protein, steroids, anthroquinonen, phenols, terpenoids and carbohydrates [46]. They found out the plant extract of *Phyllanthus niruri* showed presence of phenol, flavonoids, alkaloids, terpenoids and saponins with significant antioxidant property. In aqueous extracts all the phytochemicals showed highly positive except steroids, next to aqueous the methanol extract showed more number of phytochemicals some are highly positive and some are positive.

Table 2 Quantitative estimation of flavonoids Standard

Concentration (µg/ml)	O.D.
200	0.108
400	0.297
600	0.428
800	0.575
1000	0.750

Table 1 Phytochemical screening of *Phyllanthus niruri* in different solvent extracts

Test	Methanol	Acetone	Chloroform	Aqueous	Ethyl acetate
Tannin	++	-	-	+++	-
Saponin	-	++	++	+++	-
Flavonoids	++	-	++	+++	++
Alkaloids	+++	++	++	+++	+++
Proteins	+++	++	++	+++	++
Steroids	+++	-	-	++	-
Anthroquinones	+	-	-	+++	-
Phenols	+++	-	+	+++	-
Terpenoids	+++	-	+++	+++	++
Carbohydrates	+++	++	+++	+++	+++

(+) - Positive
(-) –Negative
(+++) - Highly Positive

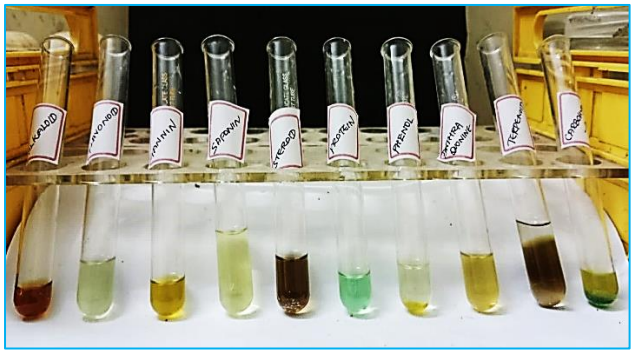


Fig 1 Qualitative phytochemical analysis of *Phyllanthus niruri* (methanol extract)

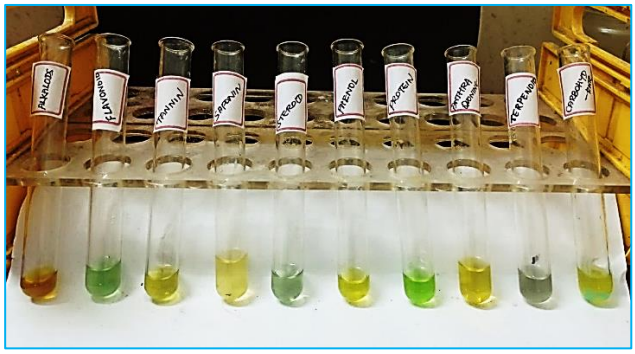


Fig 2 Qualitative phytochemical analysis of *Phyllanthus niruri* (acetone extract)

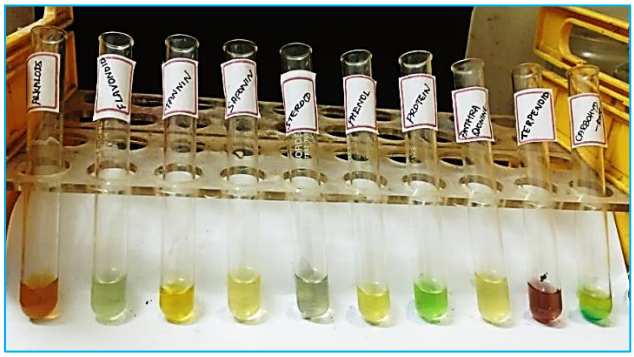


Fig 3 Qualitative phytochemical analysis of *Phyllanthus niruri* (chloroform extract)

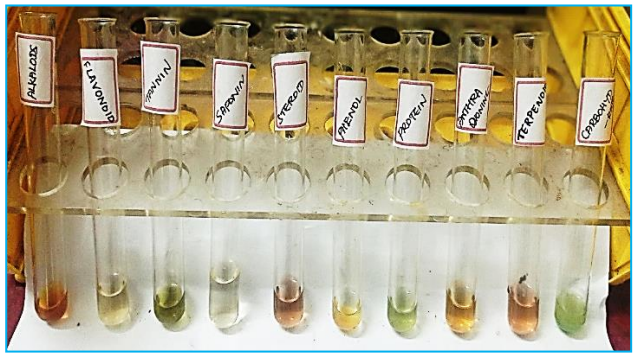
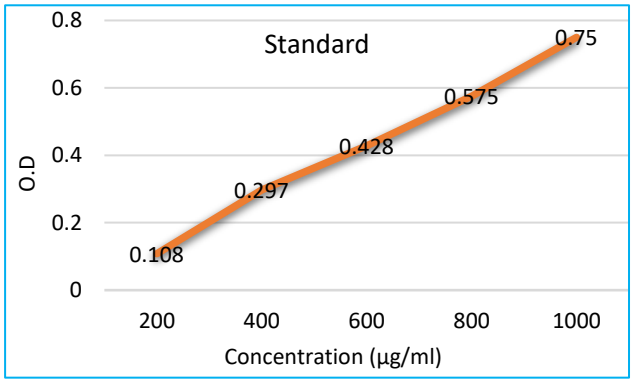


Fig 4 Qualitative phytochemical analysis of *Phyllanthus niruri* (aqueous extract)



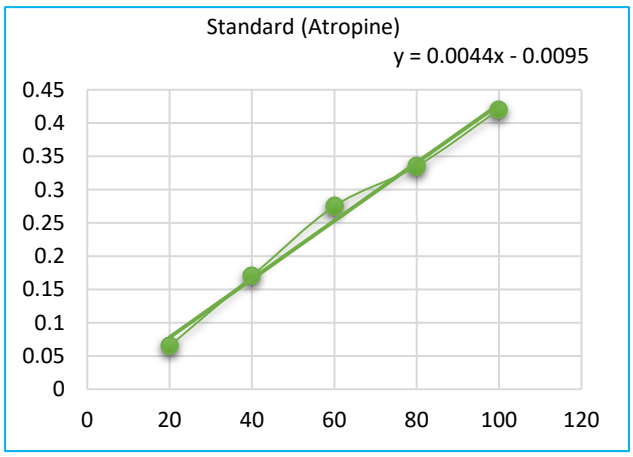
Graph 1 Quantitative estimation of flavonoids

Table 3 Total estimation of flavanoids

Sample	O.D.	Flavanoids content (mg/ml)
Phyllanthus niruri (Methanol extract)	0.158	258
Phyllanthus niruri (Aqueous extract)	0.176	275

Table 4 Quantitative estimation of alkaloids: Alkaloid content of standard (Atropine)

Concentration (µg/ml)	O.D.
20	0.065
40	0.170
60	0.275
80	0.335
100	0.420



Graph 2 Quantitative estimation of alkaloids

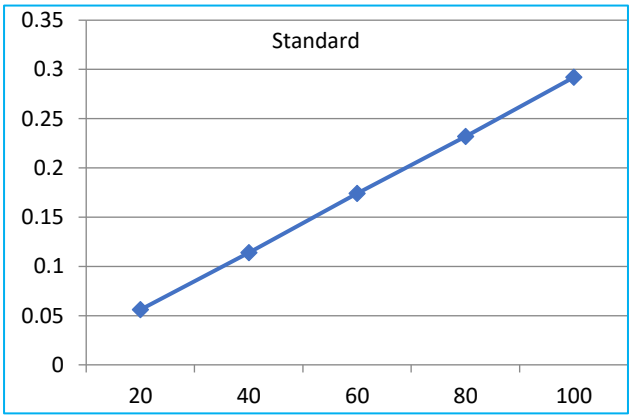
Table 5 Alkaloid content of sample

Sample	Sample weight	Dry weight (µg/mg)
Phyllanthus niruri (Methanol extract)	1mg	0.069
Phyllanthus niruri (Aqueous extract)	1mg	0.080

The quantitative estimation of alkaloids, flavonoids and phenol were carried out. The alkaloids showed 0.080 µg/ml in 1 mg of sample in aqueous extract. The flavonoids showed 275 mg/ml and phenolic content showed 158 mg/ml in 1 mg of sample in aqueous extract [46]. They reported that the phenols and Flavonoids are the secondary metabolites and have Antimicrobial and Antioxidant activity, their presence was quantitatively determined. The extract of *Phyllanthus niruri* showed the presence of phenols and Flavonoids both qualitatively and quantitatively. The study indicates the presence of Alkaloids, phenols and Flavonoids both qualitatively and quantitatively. The analysis of the total phenol content showed the presence of total phenol content equivalent to 28.05 µg of gallic acid in 1 mg of the plant extract. Flavonoids which are responsible for pigmentation in plants was also evaluated quantitatively.by the standard graph formed using quercetin, the flavonoid content 1 mg of the plant extract was found to be equivalent to 61.41 µg of quercetin. These results confirm the conclusions from the ethnobotanical study of *Phyllanthus niruri* showed more amount of phyto constituents in aqueous extract both qualitatively and quantitatively.

Table 6 Total estimation of phenolic content Standard

Concentration (µg/ml)	O.D.
20	0.056
40	0.114
60	0.174
80	0.232
100	0.292



Graph 3 Quantitative estimation ofphenolic content

Table 7 Total estimation of phenol in *Phyllanthus niruri*

Sample	O.D.	Phenol content (mg/ml)
Phyllanthus niruri (Methanol extract)	0.549	155
Phyllanthus niruri (Aqueous extract)	0.574	158

CONCLUSION

From the investigation of this study were revealed the qualitative and quantitative examination of *Phyllanthus niruri* in five different extracts Methanol, Aqueous, Chloroform, Acetone and Ethyl acetate showed predominantly presence of various bioactive components. However, Aqueous, Methanol, leaf extraction of *Phyllanthus niruri* showed high presence of most of the phytochemical components than the other solvents. It also indicates that the high content of alkaloids: flavonoids and phenol. This investigation may further open to new area of medical world and formulation of novel drugs which were present of bio active component in this plant extraction.

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