INTERNATIONAL JOURNAL OF PHARMACEUTICAL RESEARCH AND NOVEL SCIENCES

IJPRNS

EFFECT OF SOLVENTS ON TOTAL PHENOLIC CONTENT, TOTAL FLAVANOID CONTENT AND *IN VITRO* ANTIOXIDANT PROPERTIES OF *PHYLLANTHUS NIRURI*

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ABSTRACT

The study aimed to evaluate the effect of different solvents in estimation of total phenolic content, flavanoid content and antioxidant properties of *Phyllantus niruri*. *Phyllantus niruri* as a whole plant is a popular folkfore medicine and are used in the treatment of various diseases especially in hepatitis and other viral infection. Phenolic content of extracts were determined using Folin-Ciocalteu assays, total flavanoid content was estimated by vanillin reagent method and *invitro* antioxidant activity was carried out by DPPH method. The results showed that different solvent with different polarity possess significant effect on total phenolic, total flavanoid and antioxidant activities.

KEY WORDS- *Phyllanthus niruri*, Total phenolic content, Total flavanoid content, DPPH

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INTRODUCTION

Phyllanthus niruri is a herb belongs to the family of Euphorbiaceae and and grows upto 60 cm in height. The plant is originated in India, and usually occurring as a winter weed throughout the hotter parts (1). The *Phyllanthus* genus consists of 600 species of shrubs, trees and annual or biennial herbs which is distributed throughout the tropical and subtropical areas. Phyllanthus means “leaf and flower” because the flower, as well as the fruit, seems to become one with

the leaf. This plant is very popular in folkfore medicine, as whole plant which consist fresh leaves and fruits are used in the treatment of various diseases especially in hepatitis and other viral infection (2). It is well known for its liver healing properties and so used in Chinese medicine for treatment of liver diseases. It is used in Brazilian folk medicine by patients with urolithiasis. In ayurvedic medicine the herb is used for treating gastric lesion, urolithiasis, jaundice, urinary infections, diuretics, bronchitis, leprosy, anaemia, asthma and other liver related disorders. According to the Unani System of medicine this herb is good for chronic dysentery. Its seeds are used in the treatment of ulcers, wounds, scabies and ringworms. The root of this plant is considered to be an excellent remedy for liver diseases (3-5).

It have been reported that phyllantus possess antiinflammatory property by decreasing the inflammation caused by several pathways. *Phyllanthus niruri* has been reported to exhibit marked antihepatitis B virus surface antigen activity in in-vivo and in-vitro studies (6). The herb showed hypolipedemic activity in triton induced hyperlipidaemia in rats. An alcoholic extract of *Phyllanthus niruri* was found to reduce significantly the blood sugar in normal rats and in alloxan diabetes rats. The herb also showed antimalarial, antispasmodic and analgesic activities (4, 7). The active phytochemicals include flavonoids, alkaloids, terpenoids, lignans, polyphenols, tannins, steroids, coumarins and saponins, have been identified from various parts of *P. Niruri.* The isolated phytoconstituents includes ricinoleic acid, niruside, phylitate, phyltetralin, 1-o-galloyl-6-o-luteoyl-a-Dglucose,glucogallin, gallic acid, quercetin 3-O-beta-d-glucopyranosyl-(2-1)-O-beta-d-xylopyranoside,b-sitosterol,4-methoxy-nor-securinine, arabinogalactan, ellagic acid, brevifolin carboxylic acid and ethyl brevifolin carboxylate,cubebin (8,9).

Secondary metabolites from plants, mainly phenolics and flavanoids having antioxidants, antimicrobial, antitumour, antiviral, enzyme inhibiting and radical scavenging properties (10, 11). The isolation of antioxidant compounds from plants is possible through only extraction techniques with different solvents and it depends on the nature of extracting solvents because these secondary metabolites have the different solubility's in different solvents. Hence to identify a suitable solvent to extract the maximum phenolic and flavanoids content is of great priority. In this present study different solvents ranging from polar to non polar such as water, ethanol, chloroform, ethyl acetate and n-Hexane were used for preparation of extracts of *Phyllantus niruri*.

MATERIALS AND METHODS

Chemicals

Folin-Ciocalteau reagent, DPPH, Gallic acid, Ascorbic acid, Rutin, Vanilin, Sodium carbonate, DMSO, Methanol.

Collection and identification of leaf

The whole plant was collected from West Godawari District, Andhra Pradesh, India. The leaves were identified and authenticated in Department of Botany, Acharya Nagarjuna University, Guntur, Andhra Pradesh, India and a specimen (crrcog/675/2014) was preserved.

Preparation of different extracts

The leaves were air dried for 7 days and grind to coarse powder and then extracted with different solvents such as water, ethanol, chloroform, ethyl acetate and n-hexane using soxhlet extraction until colorless siphonings. The extract was freed from solvent by evaporation under reduced pressure to obtain a thick residue. Preliminary phytochemical tests were performed with the different extracts.

Determination of Total phenolic contents

The total phenolic content was determined by Folin-Ciocalteau colorimetric method. A small quantity (2 ml) of extracted solution (100 µg/ml) was mixed with 1.5 ml of Folin-Ciocalteau reagent (20% v/v). After 4 min, 4ml Na2CO3 (7%) was added and volume was made up to 10ml by water. The mixture was allowed to stand for 90 min, protected from light at room temperature. The absorbance was measured at 760nm by using Shimadzu UV/Vis 2401 spectrophotometer. The phenolic content was calculated using calibration curve of Gallic acid (20-100 µg/ml). The result is expressed as mg of Gallic acid/g dry material (12).

Determination of Total flavanoidal content

The total flavanoids are estimated by vanillin reagent. An aliquot (1 to 10 ml) of extracts were added into respective test tubes. To these solutions 4ml of vanillin reagent was added and the volume is made to 14 ml

with distilled water. The test tubes are heated for 15 minutes in a boiling water bath. The absorbance was measured by using Shimadzu UV/Vis 2401 spectrophotometer. The flavanoidal content was calculated using calibration curve of rutin (10-100 µg/ml) by dissolving rutin (100mg) in 100 ml of methanol. The result is expressed as rutin equivalent in mg/g of the extract (13).

***In vitro* antioxidant activity**

The extracts were tested for *in vitro* antioxidant activity using four standard methods. The concentrations of the sample and standard solutions used were 1000, 500, 250, 125, 62.5, 31.25, 15.625, 7.812, up to 0.025 µg/mL. The absorbance was measured spectrophotometrically against the corresponding blank solution. The percentage inhibition was calculated by using the following formula.

$$\text{Radical scavenging activity} = \frac{\text{OD control} - \text{OD sample}}{\text{OD control}}$$

Radical scavenging activity by DPPH method

2 , 2 - diphenyl - 1 - picryl – hydazyl (DPPH) is widely used to test the ability of compounds as free radical scavengers or hydrogen donors. Samples of different concentrations were prepared by dissolving the oil in DMSO. 100 µL of either the samples or the standard solution was taken separately in test tubes and 2 ml of DPPH solution (0.1 mM) was added. The test tubes were incubated at 37ºC for 30 min and the absorbance of each solution was measured at 490nm (14). A control in these experiments was prepared by same protocol except that the compound was not included.

RESULTS AND DISCUSSION

Effect of solvent on identification of phytoconstituents

The preliminary qualitative phytochemical studies were performed and the results are shown in Table -1. The result shows the presence of phenols and flavanoids in all the extracts. But compounds like steroids, alkaloids are not present in all the extract as these secondary metabolites have the different solubility's in different solvents.

Table-1 Qualitative phytochemical analysis of different extracts of *Phyllanthus niruri*

Tests	Types of extract				
	Aqueous	Ethanol	Chloroform	Ethyl acetate	n-Hexane
Carbohydrates	+	+	+	+	-
Phenols	+	+	+	+	+
Flavanoids	+	+	+	+	+
Alkaloids	+	+	+	-	-
Steroids	-	+	+	+	+
Glycosides	+	+	+	-	-
Proteins	+	+	+	+	+
Fixed oil	-	-	-	-	-

Effect of solvent on estimation of Total phenolic content

The total phenolic content was determined in all the five extracts and the results are shown in **Fig-1**. The amount of phenols is found to be more in ethanol, water followed by chloroform. The ethanolic extract showed a highest concentration of 79.2mg in 400 mg of extract. The ethyl acetate and n-hexane solvent extracts showed a very minimum concentration of phenolic content such as 39.1mg and 13.2mg respectively in 400mg of extract. These results suggested that the extractability of phenolic compounds is influenced by the polarity and viscosity of the solvents used.

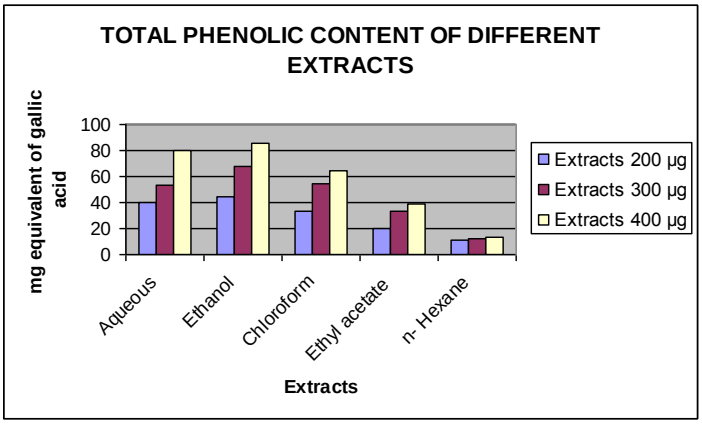

Fig-1 The Total phenolic content of different extracts of *Phyllanthus niruri*

The total phenolic content was determined by the Folin-Ceucalteau procedure which is convenient, simple, and reproducible. The mechanism behind the Folin-Ceucalteau procedure is involved the reduction of the molybdenum component in the phosphotungstic -phosphomolybdic complex reagent (12). Phenolic are a group of phytochemical compounds present in the

plant often with other molecules like proteins, polysaccharides, terpenes, flavanoids, chlorophyll and inorganic compounds. Polyphenolics essentially represents as natural antioxidants (15). They include flavanoidal polyphenols, anthocyanins, catechins, phenolic acids etc…As it is present along with many other constituents a suitable solvent is necessary to extract phenolic compounds. The results shows that the amount of phenolics are more in ethanol and water clearly indicating that phenols require more polar organic solvents.

Effect of solvent on estimation of total flavanoid content

The total flavanoid contents were determined in all the five extracts and the results are shown in **Fig-2.** The amounts of flavanoids are more in water, ethanol and chloroform when compared with the other solvents. A highest concentration of 75.3mg and 79.2 mg was observed in 400 mg of aqueous ands ethanolic extracts. The highly non-polar solvent n-Hexane showed a least concentration of 2.2mg in 400 mg of extract.

Flavanoids are flavone like substances with good antioxidant and anti-inflammatory properties (10). The result shows the flavanoid are rich in polar solvents than non polar solvents hence polar solvents are the suitable solvent system for extraction and estimation of flavanoids of *Phyllanthus niruri.*

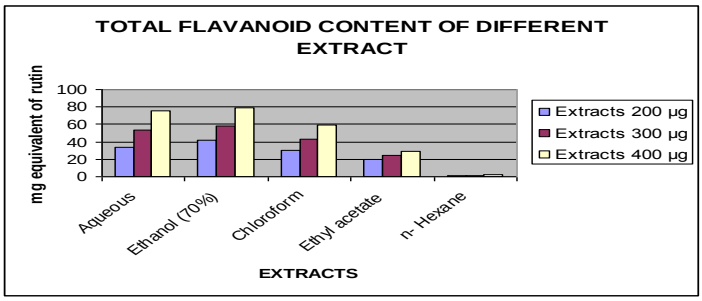

Fig -2 The total flavanoid content of different extracts of *Phyllanthus niruri*

Effect of solvent in *In vitro* antioxidant properties

The *In vitro* antioxidant screening was performed by standard DPPH method for all the extracts. The results of antioxidant activities are shown in **Table-2.** In DPPH method the water, ethanolic and chloroform extract showed the IC_{50} value at lowest concentrations of 12.5, 8.19 and 18.30 respectively when compared with standard. The ethyl acetate and n-Hexane extracts showed the IC_{50} value at higher concentrations.

Solvent used for extraction of *Phyllanthus niruri* have significant effect on its antioxidant activity. The potent antioxidant activity of aqueous, ethanolic and chloroform may achieved by the presence of high concentrations of phenol and flavanoids present in these extracts. Antioxidant and antimicrobial properties are responsible for well being of human body hence, they are very much important for further characterization of plant material (16). Secondary metabolites from plants, mainly phenolics, flavanoids having antioxidants, antimicrobial, antitumour, antiviral, enzyme inhibiting properties. Flavanoids exert their activity by carefully scavenging the free radicals to a stable radical. It has been widely accepted that efficacy of the extraction yield and the antioxidant properties largely depend on nature of the solvents as different antioxidant compounds and phenolics respond differently towards the solvent.

Table-2 *In vitro* Antioxidant activity of different extracts of *Phyllanthus niruri*

Sample/standards	IC_{50} value ± SE (µg/mL) by methods *
	DPPH
Water	12.5±0.32
Ethanol (70%)	8.19± 0.02
Chloroform	18.30±0.08
Ethyl acetate	20.08 ±0.42
n-Hexane	54.56±0.22
Ascorbic acid	0.85±0.35

***Average of three determinations**

CONCLUSION

Extraction with different solvents affect the total phenolic content, total flavanoidal content and antioxidant properties of *Phyllanthus niruri*. Regardless of other solvent used, the most efficient solvent for extraction of phenolic and flavanoidal content is ethanol (70%) which also showed good free radical scavenging activity. In conclusion the present study clearly demonstrated that extraction solvent greatly affected the extraction of phenolic, flavanoidal as well as the antioxidant activity of *Phyllanthus niruri*

ACKNOWLEDGEMENT

We are thankful to Sir C R Reddy College of Pharmaceutical Sciences for their constant support and providing all necessary requirements to carry out this work.

REFERENCES

1. Rastogi RP, Mehrotra BN. Compendium of Indian medicinal plants, Vol. II. Central Drug Research Institute, Lucknow and publications and Information Directorate, New Delhi, 1991.
2. Bagalkotkar G, Sagineedu S, SaadJ MS. Phytochemicals from *Phyllanthus niruri* Linn. and their pharmacological properties, *J Pharm Pharmacol* 2006; 58:1559- 7150.
3. Chauhan JS, Sultan M, Srivastava SK. Two new Glycoflavones from the roots of *Phyllanthus niruri, Planta Med.* 1977; 32: 217-222.
4. Chandra R. Lipid lowering activity of *P. niruri*, *Journal of Medicinal & Aromatic Plant Sci* 2000; 22: 29-30.
5. Kitisin T. Pharmacological studies III Phyllanthus niruri, *Siriraj Hospital Gaz.* 1952; 4:641-649.
6. Meixa W, Yanjin C. Herbs of the genus Phyllanthus in the treatment of Chronic hepatitis B observation with three preparation from different geographic sites. *J Lab Clin Med* 1995; 126: 350.
7. Raphael KR, Sabu MC. Antidiabetic activity of *Phyllanthus niruri*, *Amala. Res Bull* 2000; 20: 19-25.
8. Matsuura Subeki H, Takahashi K, Kobayashi S. Trimurningsih,C.:Antibabesial and anti-plasmodial compounds from *Phyllanthus niruri*. *J Nat Prod* 2005; 68: 537-539.
9. Thyagarajan SP, Subramanian S. Effect of *Phyllanthus niruri* on chronic carriers of hepatitis B virus. *Lancet.* 1988; 2:7646.
10. Sineiro J, Franco D, Rubilar M, Sánchez M, Jerez M. Polyphenols from plant materials: extraction and antioxidant power. *EJEAF Che* 2008; **7**: 3210-3216.
11. Sengul M, Yildiz H, Gungor N, Eser Z and Ercisli S. Total phenolic content, antioxidant and antimicrobial activities of some medicinal plants. *Pak J Pharm Sci* 2009; 22: 102-106.
12. Ikram EHK, Eng K, Jalil AMM, Ismail A, Idris A. Antioxidant capacity and total phenolic content of Malaysian underutilized fruits, *J Food Comp Anal* 2009; 22**:** 388-393.
13. Soumya V, Venkatesh P, Shrishaillapa B. A novel microwave-assisted extraction for the isolation of andrographolide from *Andrographis paniculata* and its *in vitro* antioxidant activity. *Nat Prod Res* 2010; 24: 1560-1567.
14. Aguilera-Carbo A, Augur C, Lilia A, Barragan P, Favela-Torres E. Microbial production of ellagic acid and biodegradation of ellagitannins. *Appl Microbiol Biotechnol* 2008; 78**:** 189-199.
15. Khanavi M, Hajimahmoodi M, Cheraghi-Niroomand M, Kargar Z, Ajani Y. Comparison of the antioxidant activity and total phenolic contents in some *Stachys* species. *Afr J Biotechnol* 2009; 8: 1143-1147.
16. Bushra S, Farooq A,Muhammad A. Effect of extraction solvent/technique on the antioxidant activity of selected medicinal plant extracts. *Molecules* 2009; 14: 2167- 2180.