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Metabolic fingerprinting of root, stem and leaf extracts of *Phyllanthus amarus*

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ABSTRACT

Phyllanthus amarus belonging to family Euphorbiaceae is an important medicinal plant from ancient times which is used in Chinese and Ayurvedic medicine. It is a small annual herb mostly found in Central and Southern India. All parts of this plant have medicinal value and are used in traditional medicines for curing various diseases such as jaundice, hepatitis B, C, microbial infections, viral diseases, tumors, and kidney stones. Keeping in view its immense medicinal potential, phytochemical analysis was performed using different solvents such as ethyl acetate, dimethylformamide, chloroform, dichloromethane, and n-Hexane. All the plant parts, *viz.,* root, stem and leaf were separately analyzed for the detection of phenols and flavonoids, of which leaf exhibited the highest concentration compared to root and stem. Maximum percentage of phenols and flavonoids could be detected with dimethylformamide in comparison to other solvents that were used in the study. Dimethylformamide leaf extract displayed highest phenolic content of 40.75 mg/g and the highest flavonoid content of 51.5 mg/g.

KEY WORDS: Flavonoids, phenols, *Phyllanthus amarus*, phytochemical

INTRODUCTION

India is one of the richest biodiversity centers in the world with different types of plant species. This huge diversity is due to different agro-climatic conditions throughout. Every higher plant species has got its own metabolic fingerprint with secondary metabolites of pharmaceutical value, which is used in traditional and modern medicines. Herbal products are safe to human and environment in contrast to the synthetic drugs (Panda *et al.*, 2012). In India, plant drug contribution is as much as 80% compared to developed countries (Babu *et al.*, 2011). Traditional systems of medicine are widely practiced due to high population, high treatment cost, side effects of synthetic drugs, insufficient drug supply, and development of resistance to synthetic drugs. In the recent past, the herbal system of medicines has reached a very vital phase. Green plants are used as raw materials as they possess a variety of compounds, which can be extracted and can be used for a variety of scientific investigations. Secondary metabolites present in plant are commercially important in pharmaceutical industries (Joy *et al.*, 2001).

Phyllanthus amarus, commonly called as Bhui Amla, belonging to family Euphorbiaceae, is a small herb in southern India with great medicinal value (Nair and Abraham, 2008), growing to a height of 50-70 cm, bearing herbaceous branches with smooth and light green bark. It has numerous pale green flowers and the fruits are tiny, smooth capsules containing seeds. *P. amarus* root and leaf extract showed significant inhibition of hepatitis C virus (Ravikumar *et al.*, 2011). Leaves, roots, stem, bark and berries of this genus contain lignans (e.g. phyllanthin and hypophyllanthin) and a variety of other phytochemicals such as alkaloids, flavonoids (e.g. quercetin), glycosides, ellagitannins, and phenylpropanoids (Bagalkotkar *et al.*, 2006). Common lipids, sterols, and flavonols also occur in the plant which makes it an unusual choice for innovating phytopharmaceuticals. The objective of this study is to analyze and quantify phenols and flavonoids in different parts of *P. amarus* (root, stem and leaf) using different solvents.

MATERIALS AND METHODS

Collection of Plant Material

Plants of *P. amarus* were collected from fields of Andhra Pradesh, India, and authenticated by Dr. S. B. Padal, Associate Professor, Department of Botany, Andhra University.

Sample Preparation

Fresh plant material was washed under running tap water and separated into three different parts, i.e., stem, root and leaves. They were dried under shade to remove moisture and homogenized to fine powder and stored in air tight bottles. The solvents used for the extraction were ethyl acetate, dimethylformamide, dichloromethane, chloroform, and n-Hexane.

About 1 g powder of all plant parts (root, stem, leaves) was weighed separately and ground well. Dried powder was extracted in different solvents in a conical flask and kept on a rotary shaker at 190-220 rpm for 24 h. The filtrates were concentrated under reduced pressure using a Rota Vapor (IKA, Germany).

Phytochemical Screening

The preliminary phytochemical tests on different extracts were performed by specific reagents using standard phytochemical methods.

Estimation of Total Phenolic Content

Total phenolic content was estimated by the procedure of Singleton and Rossi (1965) and McDonald *et al*. (2001).

Preparation of Standard Solution

Tannic acid was used as standard for estimating the total phenolic content in *P. amarus* (0.1 mg of tannic acid was taken in 1 ml of methanol to get 0.1 mg/ml standard solution).

Preparation of Calibration Curve

Folin–Ciocalteu reagent method was used for the determination of phenolic content in plant extracts using spectrophotometric analysis (Singleton and Rossi, 1965; McDonald *et al*. 2001). Different aliquots of tannic acid (50, 100, 150, 200, and 250 µl) were taken from the stock solution (0.1 mg/ml) in test tubes. To that 5 ml of Folin–ciocalteu reagent was added and mixed well. Later 4 ml of sodium carbonate was added and all the test tubes were vortexed for 50 s. All the test tubes were kept for 30 min in water bath at 40°C. Total phenolic content in the plant extract was estimated using different aliquots of tannic acid as standard. The color developed was measured at 680 nm using UV-VIS spectrophotometer (Elico, India). Blank was prepared with Folin–Ciocalteu reagent and sodium carbonate without plant extract or tannic acid. A graph was plotted by taking the optical density values on Y-axis and concentration of tannic acid on X-axis (Figure 1).

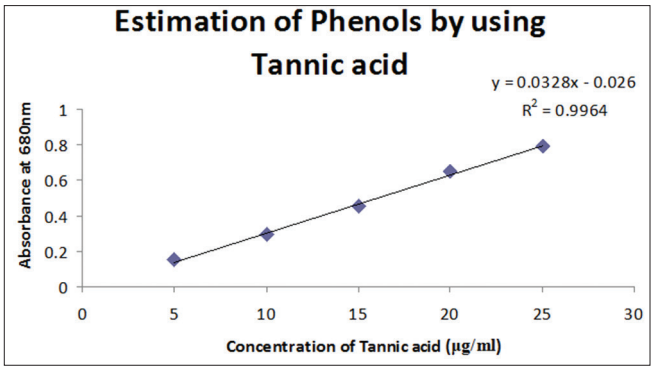

Figure 1: Standard graph for tannic acid

Preparation of Test Sample Solution

To 1 ml of the plant extract, 5 ml of FC reagent and 4 ml Na_2CO_3 were added and incubated for 30 min at 40°C. OD values were taken at 680 nm. Quantification of phenols was done for root, stem and leaf extracts of *P. amarus* based on the absorbance measured and by comparing the results with a standard curve of tannic acid. Phenol concentration was estimated from the standard curve in root, stem and leaf extracts separately and was expressed in terms of tannic acid (mg of tannic acid/g of extract).

Estimation of Total Flavonoid Content

Total flavonoid content was estimated by the procedure of Satishkumar *et al*., 2008; Patel *et al*., 2010; Patel *et al*., 2012; Pallab *et al*., 2013.

Preparation of Standard Solution

Quercetin was used as standard for estimating the total flavonoid content of the plant *P. amarus* (1 mg of quercetin was dissolved in 1 ml of methanol and was taken as standard to get 1 mg/ml solution).

Preparation of Calibration Curve

Quercetin method was used for the determination of total flavonoid content in plant extracts using spectrophotometric analysis (Satishkumar *et al*., 2008; Patel *et al*., 2010; Patel *et al*., 2012; Pallab *et al*., 2013). Different aliquots of quercetin (15, 30, 60, 90, 120, 150, 180, and 210 µl) were taken from the stock solution (1 mg/ml) in test-tubes. To those respective dilutions of methanol was added and made the volume up to 1500 µl and mixed well. Later 0.1 ml of 2% aluminum chloride and 0.1 ml of 1 M potassium acetate was added and mixed well. All the test-tubes were allowed to stand for 30 min at room temperature. Blank was prepared with aluminum chloride and potassium acetate without plant extract or quercetin. Absorbance was measured for the color that was

developed at 420 nm using UV-VIS spectrophotometer. A graph was plotted by taking the optical density values on Y-axis and concentration of quercetin on X-axis (Figure 2).

Preparation of Test Sample Solution

To 1 ml of the plant extract, 0.5 ml of $AlCl_3$ was added to the plant extract and incubated for 1 h at room temperature. To that 0.1 ml of potassium acetate (CH_3COOK) was added and mixed well. Quantification of flavonoids in root, stem and leaf extracts of *P. amarus* was done based on the absorbance measured at 420 nm and by comparing the results with standard curve of quercetin. Flavonoid concentration was estimated from the standard curve in root, stem and leaf extracts separately and was expressed in terms of quercetin (mg of quercetin/g of extract).

Calculation

Test concentration = OD of Test/Dilution Factor.

Dilution Factor = OD of Standard/Concentration of Standard.

RESULTS AND DISCUSSION

Phenols and flavonoids in different parts (root, stem and leaf) of *P. amarus* were evaluated in different solvents, i.e., ethyl acetate, dimethylformamide, chloroform, dichloromethane and n-Hexane (Shah and Yadav, 2015). Leaf was found to possess the highest activity of phenols and flavonoids compared to root and stem. Dimethylformamide was found to be the best solvent for extraction of phenols and flavonoids in root, stem and leaf extracts among the solvents tested. Dimethylformamide leaf extract exhibited highest phenol percentage of 46.77% (Figure 3) and highest flavonoid percentage of 56.18% (Figure 4).

There was report on qualitative and quantitative phytochemical screening of different plant parts of *P. amarus* with water, methanol, ethyl acetate and petroleum ether (Awasthi *et al.*, 2015). Similar studies have been reported in *Phyllanthus fraternus* (Kavit *et al.*, 2013) and different species of *Phyllanthus* leaf extracts for preliminary phytochemical evaluation (Gopinath *et al.*, 2012).

Gas chromatography-mass spectrometry (GC-MS) analysis of *P. amarus* in dimethylformamide leaf extract revealed the presence of compounds displayed in Table 1 (Figure 5). Carissanol dimethyl ether and fumaric acid were detected in maximum concentration by GC-MS analysis. Fumaric acid has been reported to possess exceptional anti-

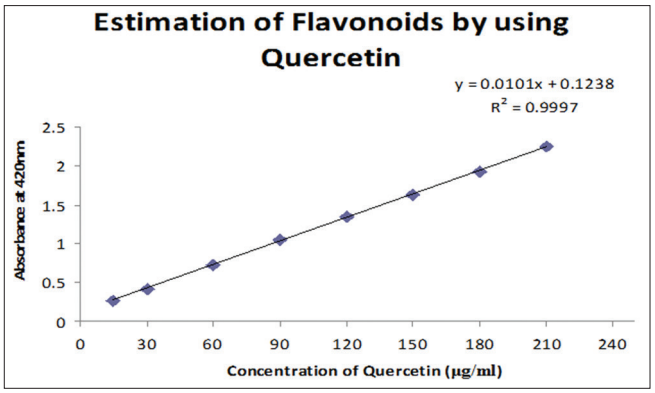

Figure 2: Standard graph for quercetin

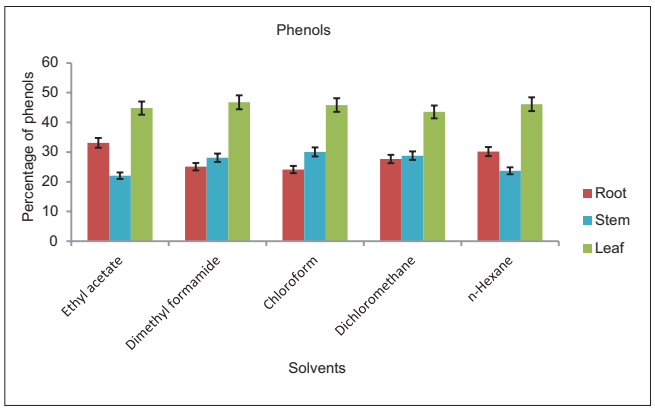

Figure 3: Comparative study of phenols in different parts of *Phyllanthus amarus* using different solvents

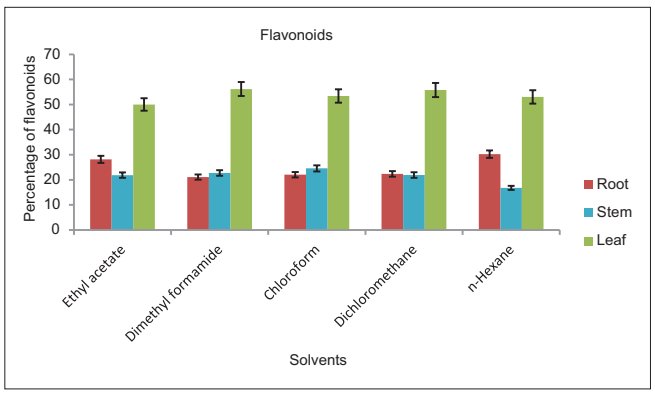

Figure 4: Comparative study of flavonoids in different parts of *Phyllanthus amarus* using different solvents

microbial and antioxidant properties. Hence, it was mostly used in a number of industrial processes (Huan *et al.*, 2002). Our reports are in corroboration with the reports of Veena Gayathri in *Tridax procumbens* (Krishnaswamy and Christina, 2015) and Dib *et al.*, (2013) in *Arbutus unedo*. Sylvatesmin detected in this extract was reported to have high radical scavenging activity (Saha *et al.*, 2015) and the compound 1-Heptacosanol was reported to have anti-microbial, anti-oxidant and nematicidal properties in some

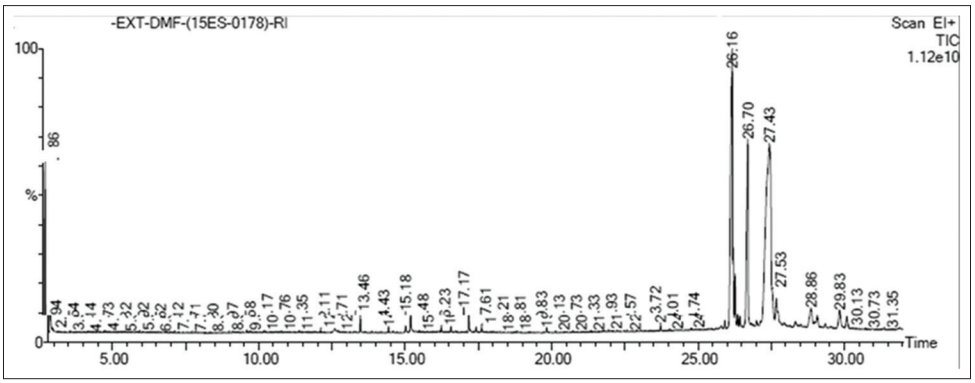

Figure 5: Gas chromatography-mass spectrometry chromatogram of dimethyl formamide leaf extract of *Phyllanthus amarus*

Table 1: List of compounds detected by GC-MS analysis in *Phyllanthus amarus* dimethylformamide leaf extracts

Rt (min)	Compound name	CAS
2.863	Formamide, N,N-Dimethyl-	68-12-2
26.163	Carissanol dimethyl ether	41328-80-7
26.203	No library	
26.273	No library	
26.703	No library	
27.434	Sylvatesmin	487-39-8
27.674	Fumaric acid, 2-isopropylphenyl pentadecyl ester	900344-90-4
28.869	Phenethylamine, 2-methoxy-. alpha.-methyl-4,5-(methylenedioxy)-	23693-18-7
29.845	1-Heptacosanol	2004-39-9

GC-MS: Gas chromatography-mass spectrometry, RT: Retention time

marine algae (Murugan and Iyer, 2014). Methoxy group containing compounds have been reported to exhibit antimicrobial action by distorting cell surface (Baluja *et al*., 2015). GC-MS analysis of our present study also revealed the presence of methylenedioxy compound which could be liable for anti-microbial activity.

CONCLUSION

Medicinal and aromatic plants comprise the basis of primary health care for bulk of the population and are an important source of income for rural population. Ancient traditional systems of medicine are practiced in Asia mainly because of historical circumstances and cultural beliefs. Medicinal plants are accessible, affordable and culturally appropriate source of primary health care for more than 80% of the Asian population according to the World Health Organization. There is a need for harmonization among various institutes of the region working on medicinal plants and traditional medicines to develop drugs for health care (Swami *et al*., 2006). The whole plant of *P. amarus* was best suitable for herbal medicine because of many active constituents present in it and opens a gate way for novel drug discovery.

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