**Scholars Academic Journal of Biosciences (SAJB)**
***Abbreviated Key Title: Sch. Acad. J. Biosci.***
**ISSN 2347-9515 (Print)**
**ISSN 2321-6883 (Online)**

A Unit of Scholars Academic and Scientific Society, India
www.saspublishers.com

Pharmaceutical Science

# Comparison of Total Phenolic Content and Anti-Oxidant Activity of Hydro-ethanolic and Chloroform Stem Extracts of *P. polyphyllus*

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**Original Research Article**

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**Article History**
*Received: 03.11.2018*
*Accepted: 05.12.2018*
*Published:30.12.2018*

**DOI:**
10.36347/sajb.2018.v06i12.003

**Abstract:** The search for antioxidant compounds from natural sources is gaining importance. The study aims to determine the total phenolic content and compare the *in-vitro* anti-oxidant activity of the hydro-ethanolic and chloroform stem along with the bark extracts of *Phyllanthus polyphyllus*, a plant having medicinal properties. The total phenolic content was determined by using Folin-Ciocalteu's reagent, while the antioxidant potential was assayed by 1,1-diphenyl-2-picryl-hydrazyl (DPPH) free radical scavenging method. The chloroform extract was found to have higher phenolic content (47.778 mg/g GAE) than the hydro-ethanolic extract (28.333 mg/g GAE). The DPPH free radical scavenging activity was higher for the chloroform extract with $IC_{50}$ value of 174.38 µg/mL than the hydro-ethanolic extract with $IC_{50}$ value of 221.32 µg/mL. As the literature had reported a positive co-relation of phenolic content with anti-oxidant activity, the higher antioxidant activity of the chloroform extract might be due to higher phenolic content.

**Keywords:** *Phyllanthus polyphyllus*, Stem, DPPH, TPC, GAE.

## INTRODUCTION

*Phyllanthus polyphyllus* is a shrub to small deciduous tree reaching up to 3-6 m in height belonging to the family Phyllanthaceae. The bark is woody, smooth and brownish grey in colour. It grows in India and Sri Lanka. In India, mainly found in peninsular region. It is widely distributed in all districts of Tamil Nadu and Kollam district of Kerala [1]. The genus Phyllanthus has been reported to have over 1,000 species distributed worldwide. Many of the species of this genus have been used indigenously for the treatment of a variety of ailments for generations [2].

Many of the species are used as traditional medicines. The extracts of the plants have been reported to be used since ancient times, for treating hypertension, diabetes, hepatic, urinary, and sexual disorders, and other common ailments. The ethnic tribes of India and other Asian countries have used the plants since ancient times, as traditional home remedies. Many of the plants of the genus Phyllanthus herbs form an integral part of Ayurveda [3]. Scientific studies have reported the medicinal importance of the plant [4-11].

Oxidative stress is related to the production of reactive oxygen species and diminished levels of antioxidant system that leads to the pathogenesis of a many diseases [12]. Antioxidants are the compounds that prevent free radical induced tissue damage. They prevent the formation of radicals, scavenge them, or by promote their decomposition [13]. Antioxidants from natural origin have potential to provide lead molecules for the development of novel drugs that may reduce the risk of serious ailments caused by free radicals. This study aims to determine the total phenolic content of the chloroform and hydro-ethanolic extracts of the stem including the bark of *Phyllanthus polyphyllus* and to compare their anti-oxidant potential.

## MATERIALS AND METHODS

### Collection of plant

The stem along with bark of *Phyllanthus polyphyllus* had been collected from Tirumala region of Andhra Pradesh and had been authentified. The plant part was dried under the shade and coarse powder was made by grinding (Figure 1).

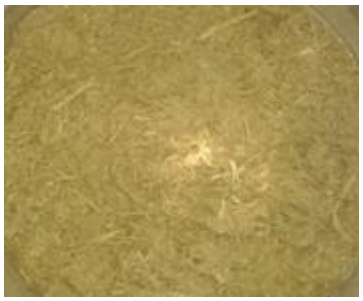

**Fig-1: Coarse powder of stem along with bark of *Phyllanthus polyphyllus***

**Extraction**

The coarse powder of the plant part was defatted with petroleum ether. Then it was subjected to extraction by using Soxhlet apparatus with chloroform followed by hydro ethanolic (Water: Ethanol= 3: 2) mixture. At once 150 g of the powdered material was introduced into the Soxhlet apparatus and each solvent was recycled for around 20 cycles. The extracts were air dried. Namely two extracts Hydro ethanolic Extract (HEPP) and Chloroform Extract of *Phyllanthus polyphyllus* (CEPP) were obtained (Figure 2).

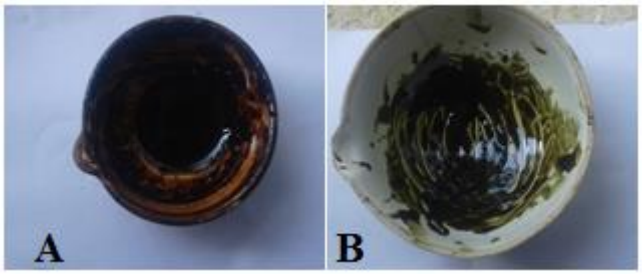


**Fig-2: Hydro alcoholic extract (A) and Chloroform extract (B)**

**Preparation of the extracts solutions**

For the determination of total phenolic content and antioxidant activity, the stock solutions of the extracts were prepared (Figure 3). For the hydro-ethanolic extract, hydro-ethanolic mixture (water: ethanol=3:2) was used as the solvent, while for the chloroform extract, ethanol was used as the solvent for dissolution. Complete dissolution was achieved by means of ultrasonication. After that dilutions were carried out accordingly.

**Fig-3: Preparation of the stock solutions of different extracts**

**Determination of total phenolic content**

Total phenolic content was determined spectro-photometrically by using Folin-Ciocalteu's reagent. The reagent is a mixture of phosphomolybdate and phosphotungstate. It is used for the *in-vitro* estimation of phenolic and polyphenolic compounds [14]. It is a non-specific method that detects all phenolic groups found in extracts.

The reaction mixture was prepared by mixing 1mL (1mg/mL) of extract, 2.5 ml of 10% Folin-Ciocalteu's reagent (in water) and 2.5 ml 7.5% $NaHCO_3$ solution (in water). After incubating at 45$^{o}$C for 45 min, the absorbance was determined using spectrophotometer at λ max = 760 nm [14]. Blank determinations were made and necessary corrections were made. Average absorbance of triplicate readings was considered.

To quantify the phenolic compounds, a calibration curve of standard anti-oxidant with increasing concentration proportional to the absorbance has to be constructed. A linear correlation equation is obtained from the curve of the type y = ax + b, where, y is absorbance and x is concentration. The correlation coefficient ($R^2$) is calculated, which represents the ability of the method to provide directly proportional results between the analyte concentration and the device response. The correlation coefficient must be equal to or greater than 0.98.

Gallic acid was used as the standard for constructing a calibration curve. Different concentrations of the gallic acid (10, 25, 50, 75, 100 µg/mL) were used. The concentration of phenolics was read from the calibration line and was expressed in terms of Gallic Acid Equivalent (mg of GAE/g of extract) [15].

$$C\ (GAE) = c \times V/M$$

Where, c = concentration determined from standard curve (mg/ml), V = volume used during the assay (ml), and M = mass of the extract used during the assay (g) and will give GAE in mg/g extract [16].

**DPPH Free Radical Scavenging Activity**

This assay procedure is the one of the most accepted models for evaluating the *in-vitro* free radical scavenging activity of a sample.

The test was performed according to the method reported by K AsokKumar *et al*. [17] with minor modification.

2.5 mL of the extract solution in ethanol (95 %) at different concentrations was added to 2.5 mL of 0.3 mM stock solution of DPPH in ethanol (95 %). 2.5 mL DPPH solution (0.3 mM) with 2.5 mL ethanol served as negative control. 2.5 mL of a solution of each concentration with 2.5 mL of ethanol served as blank. Ascorbic acid was used as standard. The samples were shaken well and kept in dark for 30 minutes at room temperature. Then, the scavenging activity on the DPPH radical was determined by measuring the absorbance at 517 nm. Correction was made for each measurement with respect to the blank reading. The DPPH radical scavenging activity (S%) was calculated using the following equation:

$$S\% = [(A_{control} - A_{sample}) / Acontrol] \times 100$$

Where, $A_{control}$ = absorbance of the blank control (containing all reagents except the extract solution) and $A_{sample}$ = absorbance of the test sample.

The IC 50 values of the extracts and standard that is the concentrations of extracts and standard required to inhibit 50% of the DPPH free radicals were calculated from the graph.

## RESULTS AND DISCUSSION

**Total Phenolic content**

The total phenolic content of the extracts was estimated in terms of gallic acid equivalent in mg/g of the extracts. The absorbance obtained for different concentrations of gallic acid (10-100 µg/ml) was plotted and calibration curve was constructed (Figure 4) using Microsoft Office Excel. $R^2$ was found to be 0.999. The total phenolic content for extracts were obtained for 1 mL (1mg/mL) of extract from calibration curve of gallic acid and the results obtained are given in Table 1. The total phenolic content of extracts were calculated using standard calibration curve (y = 0.009x - 0.018). Chloroform extract was found to have higher phenolic content (47.778 mg/g GAE) than hydro-ethanolic extract (28.333 mg/g GAE).

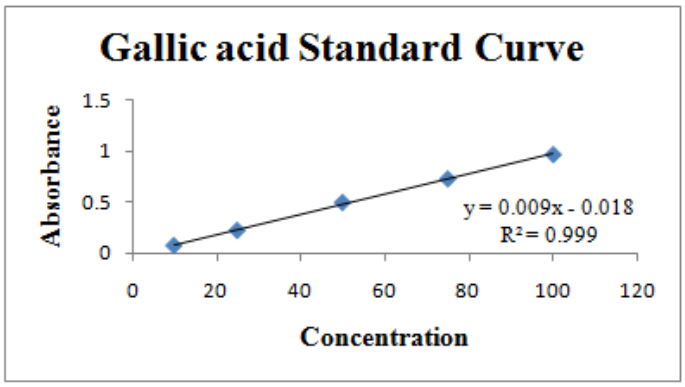


**Fig-4: Gallic acid standard curve**

**Table 1: Total Phenolic content**

| Extract | Conc. | Absorbance | | | | Conc. calculated from std. curve (µg/mL) | TPC (mg/g GAE) |
|---|---|---|---|---|---|---|---|
| | | 1 | 2 | 3 | Avg. | | |
| HEPP | 1mg/mL | 0.229 | 0.252 | 0.231 | 0.237 | 28.333 | 28.333 |
| CEPP | 1mg/mL | 0.381 | 0.454 | 0.403 | 0.412 | 47.778 | 47.778 |

**DPPH scavenging activity**

The DPPH assay method is based on the reduction of DPPH to diphenylpicrylhydrazine (DPPH-H). The 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay evaluates free radical scavenging activity by measuring the colour change that occurs when a DPPH radical is quenched by a free radical scavenger that donates a hydrogen atom or an electron. When DPPH radical accepts hydrogen atom, it gets reduced to DPPH-H and the colour of the solution converts from violet to yellow. More the decolourization of DPPH solution more is the anti-oxidant potential of the free radical scavenger [18, 19]. The DPPH free radical has maximum wavelength at 517 nm. Percentage inhibition of DPPH is calculated from the observed absorbance at this wave length after the incubation period.

The test had revealed that the both the chloroform and hydro-ethanolic extract of *Phyllanthus polyphyllus* possess DPPH free radical scavenging property. It was observed that DPPH free radical scavenging ability of both extracts increases in concentration dependent manner which was indicated by more yellow colour of the solution with higher concentration of the extracts. Therefore, after incubation the absorbance of the solutions with higher concentration of the extracts had shown lower absorbance value (Figure 5, 6).

The DPPH free radical scavenging ability of the chloroform extract was found to be higher than the hydro-ethanolic extract but compared to the standard ascorbic acid it was less. The $IC_{50}$ value of the chloroform extract and hydro-ethanolic extract were 174.38 µg/ml and 221.32 µg/ml respectively, while it was found to be 17.10 µg/ml for the standard ascorbic acid in our experimental conditions (Table 2, Figure 7).

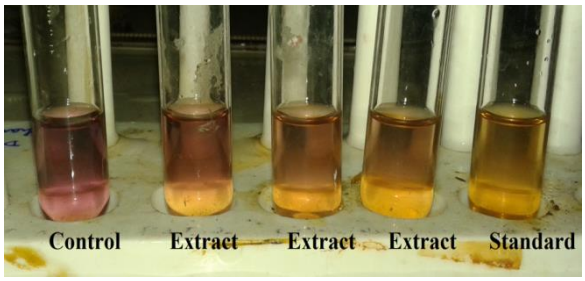


**Figure 5: After incubation of DPPH with extracts and sample**

**Table 2: Results of DPPH scavenging activity**

| Sl. No. | Concentration (µg/ml) | % Inhibition | $IC_{50}$ (µg/ml) |
|---|---|---|---|
| Ascorbic acid | | | 17.10 |
| 1 | 5 | 12.7 | |
| 2 | 10 | 25.5 | |
| 3 | 15 | 46.0 | |
| 4 | 20 | 58.1 | |
| HEPP | | | 221.32 |
| 1 | 25 | 08.41 | |
| 2 | 50 | 13.30 | |
| 3 | 75 | 19.70 | |
| 4 | 100 | 23.96 | |
| CEPP | | | 174.38 |
| 1 | 25 | 10.02 | |
| 2 | 50 | 17.84 | |
| 3 | 75 | 21.76 | |
| 4 | 100 | 31.00 | |

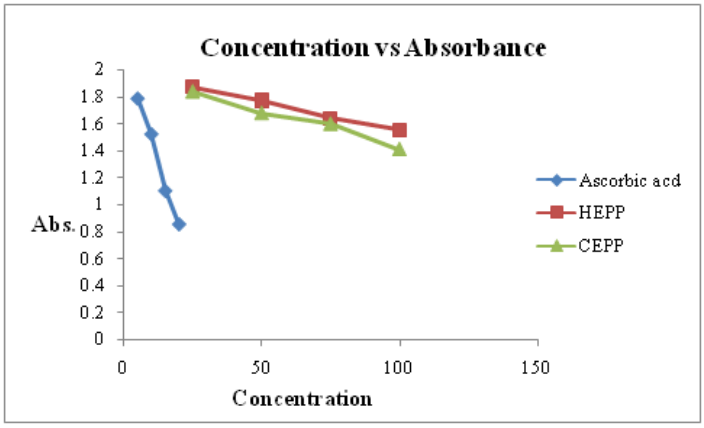


**Fig-6: Concentration versus absorbance**

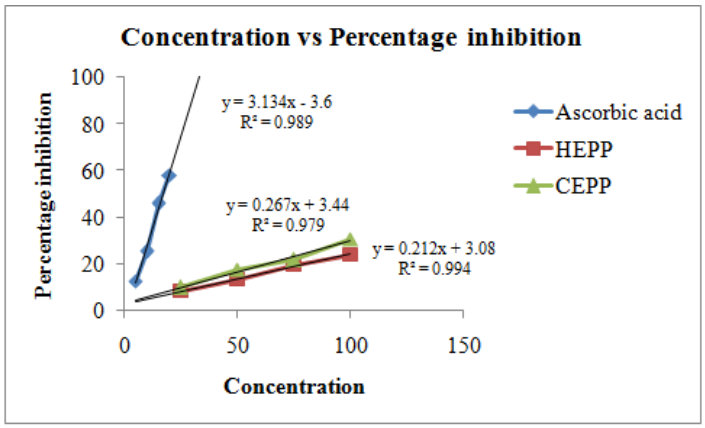


**Fig-7: Concentration versus percentage inhibition**

Phenolic compounds are the secondary metabolites in plants that characterized by at least one aromatic ring possessing one or more hydroxyl groups [20].

It has been reported that phenolic compounds possess antioxidant activity. Phenolic compounds contain hydroxyl group, they can donate hydrogen as proton and form stable phenoxyl radicals. Therefore, determination of total phenolic content is essential to determine the antioxidant capacity of herbal extracts [21]. A positive relationship between high anti-oxidant activity and phenolic content has been reported in previous studies [20, 22, 23].

Thus, it has been observed that the chloroform extract possess higher phenolic content (GAE 47.778 mg/g), which might have resulted in higher free radical scavenging activity.

## CONCLUSION

The study has suggested that both the chloroform and hydro-ethanolic stem along with the bark extracts of *Phyllanthus polyphyllus* possess antioxidant property. The chloroform extract has higher free radical scavenging ability with respect to DPPH radical than the hydro-ethanolic extract that might be due to the higher level of phenolic compounds.

**Conflict of interest:** None

**Abbreviations:**

**DPPH**: 1,1-diphenyl-2-picryl-hydrazyl, **TPC**: Total Phenolic Content, **GAE**: Gallic Acid Equivalent, **HEPP**: Hydro-ethanolic Extract of *Phyllanthus polyphyllus*, **CEPP**: Chloroform Extract of *Phyllanthus polyphyllus*, **DPPH-H**: Diphenylpicrylhydrazine

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