Assessment of phytochemicals and quantification of primary and secondary metabolites of Artabotrys hexapetalus (L.f.) Bhandari leaves

Kousalya P*1,2, Doss VA1

1Department of Biochemistry, PSG college of Arts and Science, Coimbatore-641014, Tamilnadu, India
2Department of Biochemistry, Bharathidasan College of Arts and Science, Erode - 638116, Tamilnadu, India

Abstract

The main goal of the research was to explore the existence of phytochemicals, quantification of primary and secondary metabolites of leaves extract of Artabotrys hexapetalus (L.f.) Bhandari. The phytochemical activity of leaves of Artabotrys hexapetalus was assessed using different solvent extracts like water, ethanol, acetone, chloroform and petroleum ether. Among the different solvent extracts, aqueous leaves extract revealed the high content of phytochemicals. So the aqueous leaves extract was used for further investigations. Aqueous leaves extract of Artabotrys hexapetalus was subjected to quantitative analysis of primary metabolites like carbohydrates, proteins and amino acids. Quantitative analysis of secondary metabolites like flavonoids, tannins and phenols were performed using aqueous leaves extract of Artabotrys hexapetalus. Qualitative screening of phytochemicals reported the existence of carbohydrates, amino acids, proteins, flavonoids, alkaloids, saponins, phenols, glycosides, tannins and diterpenes. Quantitative analysis showed the presence of carbohydrates (43.16±1.0 mg/g extract), proteins (60.4±0.88 mg/g extract), amino acids (19.33 ± 1.30 mg/g extract), flavonoids (28.3 ± 0.91 mg/g extract), tannins (24.53±1.02 mg/g extract) and phenols (7.63±0.85 mg/g extract). The present study concluded that aqueous leaves extract of Artabotrys hexapetalus as a potential source of phytochemicals, primary and secondary metabolites.

INTRODUCTION

Plants contain natural bioactive compounds called phytochemicals. Phytochemicals are classified into two categories primary metabolites and secondary metabolites (Krishnaiyah et al., 2009). Carbohydrates, amino acids, chlorophyll and proteins are primary metabolites. Secondary metabolites include alkaloids, flavonoids, terpenoids, tannins, phenols, glycosides, saponins (Krishnaiyah et al., 2007). The medicinal property of a plant is due to secondary metabolites (Cowan, 1999). Secondary metabolites are free radicals scavengers (Mradu et al., 2012). Analysis of phytochemicals and quantification of primary and secondary metabolites will be helpful to identify bioactive compounds. These bioactive compounds have therapeutic value in treating diseases.

*Corresponding Author
Name: Kousalya P
Phone: 9788291776
Email: kousiviswa@gmail.com

ISSN: 0975-7538
DOI: https://doi.org/10.26452/ijrps.v11iSPL4.4425

Production and Hosted by
Pharmoscope.org
© 2020 | All rights reserved.
cultivated in gardens for its very fragrant flowers. *A. hexapetalus* was used in traditional Chinese medicine to treat malaria (*Li et al., 1997*) and scrofula (*Li and Yu, 1998*). This study aims to analyse the phytoconstituents, quantification of primary metabolites (carbohydrates, proteins and amino acids) and secondary metabolites (flavonoids, tannins and phenols) in aqueous leaves extract of *Artabotrys hexapetalus*.

### MATERIALS AND METHODS

#### Plant sample collection and authentication

The leaves of *Artabotrys hexapetalus* were gathered from Erode district, Tamilnadu, India. The plant was validated (BSIS/RC/5/23/2016/Tech/2101) by Botanical Survey of India Taxonomist, Southern Regional Centre, Coimbatore, Tamilnadu, India.

#### Preparation of plant extract

Fresh leaves of *Artabotrys hexapetalus* were collected from Erode district, Tamilnadu, India. Collected fresh leaves were cleaned with sterile water and dried under shade. Dried leaves were subjected to mechanical grinding to obtain a coarse powder. The coarse powder of *A. hexapetalus* leaves (20 grams) was soaked with 200 ml of different solvents like water, ethanol (alcohol), acetone, chloroform and petroleum ether in the ratio of 1:10 for three days. Then the plant sample was extracted with a muslin cloth and used to analyse the phytoconstituents.

#### Preparation of aqueous leaves extract of *Artabotrys hexapetalus*

Large scale aqueous leaves extract, was prepared by soaking 40g leaves powder in 400ml distilled water for three days. Then the plant sample was extracted with a muslin cloth. The filtrate obtained was evaporated to dryness in a microwave oven under controlled temperature. Finally, 8 gram of greenish-brown powdered crystals were obtained, and it was kept for later analysis in tightly sealed desiccators.

### Qualitative phytochemical analysis

| S.No. | Phytochemicals         | Name of the Test                  |
|-------|------------------------|-----------------------------------|
| 1     | Alkaloids              | Dragendorff’s                      |
|       |                        | Wagner’s                           |
|       |                        | Mayer’s                            |
|       |                        | Hager’s                            |
| 2     | Flavonoids             | Alkaline reagent                   |
|       |                        | Shinoda                            |
|       |                        | Lead acetate                       |
| 3     | Saponins               | Foam                               |
|       |                        | Froth                              |
| 4     | Carbohydrates          | Fehling’s                          |
|       |                        | Benedict’s                         |
|       |                        | Molisch                            |
| 5     | Aminoacids and proteins| Ninhydrin                          |
|       |                        | Xanthoproteic                      |
|       |                        | Million’s                          |
|       |                        | Biuret                             |
| 6     | Phenols                | Ferric chloride                    |
| 7     | Glycosides             | Keller Killani                     |
|       |                        | Borntrager                         |
|       |                        | Modified Borntrager’s              |
| 8     | Tannins                | Ferric chloride                    |
|       |                        | Lead acetate                       |
| 9     | Sterols and Terpenes   | Salkowski’s (Sterols and triterpenes) |
|       |                        | Copper acetate (Diterpenes)        |
### Table 2: Qualitative phytochemical analysis

| Plant constituents | Water | Ethanol | Acetone | Chloroform | Petroleum ether |
|--------------------|------|--------|--------|------------|-----------------|
| **Alkaloids**      |      |        |        |            |                 |
| 1. Dragendorff’s   | +    | -      | -      | ++         | ++              |
| 2. Wagner’s        | ++   | +      | ++     | ++         | -               |
| 3. Mayer’s         | -    | -      | -      | -          | -               |
| 4. Hager’s         | +    | +      | -      | -          | -               |
| **Flavonoids**     |      |        |        |            |                 |
| 1. Alkaline reagent| ++   | -      | -      | -          | +               |
| 2. Shinoda         | +    | -      | -      | +          | -               |
| 3. Lead acetate    | -    | -      | -      | -          | -               |
| **Saponins**       |      |        |        |            |                 |
| 1. Foam            | ++   | -      | -      | -          | -               |
| 2. Froth           | -    | ++     | ++     | -          | -               |
| **Carbohydrates**  |      |        |        |            |                 |
| 1. Fehling’s       | +    | +      | -      | ++         | -               |
| 2. Benedict’s      | ++   | ++     | +      | -          | -               |
| 3. Molisch’s       | ++   | -      | -      | -          | +               |
| **Proteins and Aminoacids** | | | | | |
| 1. Million’s       | ++   | -      | -      | -          | -               |
| 2. Biuret          | -    | -      | -      | -          | -               |
| 3. Xanthoproteic   | +    | -      | -      | -          | -               |
| 4. Ninhydrin       | ++   | -      | -      | -          | -               |
| **Phenols**        |      |        |        |            |                 |
| 1. Ferric chloride | ++   | ++     | ++     | +          | +               |
| **Glycosides**     |      |        |        |            |                 |
| 1. Keller Killani  | ++   | -      | -      | -          | +               |
| 2. Borntrager      | -    | -      | -      | -          | -               |
| 3. Modified Borntrager’s | - | | - | - | - |
| **Tannins**        |      |        |        |            |                 |
| 1. Ferric chloride | +    | -      | -      | -          | -               |
| 2. Lead acetate    | +    | +      | +      | -          | -               |
| **Sterols And Terpenes** | | | | | |
| 1. Salkowski’s (Sterols and Trierpenes) | - | - | - | - | - |
| 2. Copper acetate (Diterpenes) | ++ | + | + | - | - |

*+ indicates the presence of phytochemicals and – indicates the absence of phytochemicals*
tive phytochemicals (Table 1) using standard procedures (Sani et al., 2007; Tiwari et al., 2011).

Analysis of total carbohydrates
Quantitative evaluation of carbohydrates in Artabotrys hexapetalus leaves was performed with anthrone reagent using a standard protocol (Hedge and Hofreiter, 1962).

Analysis of total proteins
The protein content of Artabotrys hexapetalus leaves was evaluated according to the Lowry method (Lowry et al., 1951).

Analysis of total amino acids
Standard procedure was followed to determine the amino acid content of Artabotrys hexapetalus leaves (Moore and Stein, 1954).

Analysis of flavonoids
Aluminium chloride method was used to assess the flavonoids of Artabotrys hexapetalus leaves (Woisky and Salatino, 1998).

Analysis of tannins by Folin-Denis method
The tannin content of Artabotrys hexapetalus leaves was found to be 24.53 ± 1.02 mg/g extract. Tannin plays a protective role against oxidative stress (Ness and Powles, 1997). Tannins are very effective against microorganisms and parasites. It is used in the treatment of inflammation in mouth and diarrhoea (Ofokansi et al., 2005).

Analysis of total phenols
Phenol content of aqueous extract of A.hexapetalus leaves was found to be 7.63 ± 0.85 mg/g extract. Plant phenols possess radical scavenging ability and protection against UV radiation (Bennett and Wagg, 1994).

RESULTS AND DISCUSSION
The result of phytochemical analysis of different solvent extracts of A.hexapetalus leaves has been listed in Table 2.

Among the various solvent extract of Artabotrys hexapetalus leaves, aqueous extract revealed more phytoconstituents. Carbohydrates, amino acids, proteins, alkaloids, flavonoids, tannins, diterpenes, phenols, saponins, and glycosides were reported in the aqueous leaves extract of Artabotrys hexapetalus. When compared to aqueous extract, other extracts showed fewer phytoconstituents. So the aqueous leaves extract of Artabotrys hexapetalus was taken for further studies.

Analysis of total carbohydrates
The amount of carbohydrate in aqueous leaves extract of A.hexapetalus was found to be 43.16 ± 1.0 mg/g extract (Figure 1).

Analysis of total proteins
The total protein content of aqueous leaves extract of A.hexapetalus was found to be 60.4 ± 0.88 mg/g extract.

Analysis of total amino acids
The amount of amino acid in aqueous leaves extract of A.hexapetalus was found to be 19.33 ± 1.30 mg/g extract.

Analysis of flavonoids
Flavonoid content of aqueous leaves extract of A.hexapetalus was found to be 28.3 ± 0.91 mg/g extract. Flavonoids are a potent radical scavenger and possess the metal chelating ability (Michalak, 2006; Winkel-Shirley, 2002; Rivero et al., 2001). Flavonoids possess anticancer activity, anti-inflammatory activity and lower the risk of heart disease (Okwu and Okwu, 2004).

Conclusions
Phytochemical analysis of A.hexapetalus leaves was performed using different solvent extracts like aqueous, ethanol, acetone, chloroform and petroleum ether. Among the different solvent extracts, an aqueous extract of A.hexapetalus leaves revealed the
presence of phytoconstituents like carbohydrates, amino acids, proteins, alkaloids, flavonoids, tannins, diterpenes, phenols, saponins and glycosides. Quantitative analysis of carbohydrates, proteins, amino acids, flavonoids and phenols were done using aqueous extract of *A. hexapetalus* leaves. This study concludes that aqueous extract of *A. hexapetalus* leaves is a potent source of phytochemicals, primary metabolites and secondary metabolites. Secondary metabolites act as antioxidants and scavenge free radicals. *A. hexapetalus* leaves can act as a natural antioxidant.

**Conflict of Interest**

The authors declare that they have no conflict of interest for this study.

**Funding Support**

The authors declare that they have no funding support for this study.

**REFERENCES**

Bennett, R. N., Wallsgrove, R. M. 1994. Secondary metabolites in plant defence mechanisms. *New Phytologist*, 127(4):617–633.

Cowan, M. M. 1999. Plant products as antimicrobial agents. *Clinical Microbiology Reviews*, 12(4):564–582.

Hedge, J., Hofreiter, B. T. 1962. In: Carbohydrate Chemistry, volume 17. Academic Press, New York.

Krishnaiah, D., Devi, T., Bono, A., Sarbatly, R. 2009. Studies on phytochemical constituents of six Malaysian medicinal plants. *Journal of Medicinal Plants Research*, 3(2):67–072.

Krishnaiah, D., Sarbatly, R., Bono, A. 2007. Phytochemical antioxidants for health and medicine a move towards nature. *Biotechnology and Molecular Biology Reviews*, 2(4):97–104.

Li, T., Yu, J. 1998. Studies on the chemical constituents of the leaves from Artabotrys hexapetalus. *Acta Pharmaceutica Sinica*, 33(8):591–596.

Li, T. M., Li, W. K., Yu, J. G. 1997. Flavonoids from Artabotrys hexapetalus. *Phytochemistry*, 45(4):831–833.

Lowry, O. H., Rosenberg, N. J., Farr, A. L., Randall, R. J. 1951. Protein measurement with the folin phenol reagent. *The Journal of Biological Chemistry*, 193(1):265–275.

Malik, C. P., Singh, M. B. 1980. *Plant Enzymology and Histo-Enzymology: A Text Manual*. Kalyani Publishers, New Delhi.

Michalak, A. 2006. Phenolic compounds and their antioxidant activity in plants growing under heavy metal stress. *Polish Journal of Environmental Studies*, 15(4):523–530.

Mohan, A. C. 2017. Phytochemical Analysis And Screening Of Total Flavonoid, Tannin And Phenolic Contents In Croton Bonplandianum Leaf And Stem. *World Journal of Pharmaceutical Research*, 6(4):1066–1075.

Moore, S., Stein, W. H. 1954. A modified ninhydrin reagent for the photometric determination of amino acids and related compounds. *Journal of Biological Chemistry*, 211(2):907–920.

Mradu, G., Saumyakanti, S., Sohini, M., Arup, M. 2012. HPLC Profiles of Standard Phenolic Compounds Present in Medicinal Plants. *International Journal of Pharmacognosy and Phytochemical Research*, 4(3):162–167.

Ness, A. R., Powles, J. W. 1997. Fruit and vegetables, and cardiovascular disease: a review. *International Journal of Epidemiology*, 26(1):1–13.

Ofokansi, K. C., Esimone, C. O., Anele, C. R. 2005. Evaluation of the in vitro combined antibacterial effects of the leaf extracts of Bryophyllum pinna tum (Fam: Crassulaceae) and Ocimum gratissium (Fam: Labiate). *Plant Products Research Journal*, 9:23–27.

Okwu, D. E., Okwu, M. E. 2004. Chemical Composition of Spondias mombin (Linn) plant parts. *Journal of Sustainable Agriculture and the Environment*, 6(2):140–147.

Rivero, R. M., Ruiz, J. M., García, P. C., López-Lefebre, L. R., Sanchez, E., Romero, L. 2001. Resistance to cold and heat stress: accumulation of phenolic compounds in tomato and watermelon plants. *Plant Science*, 160(2):315–321.

Sani, A. A., Mohammed, I., Kaita, H. A. 2007. Phytochemical screening of the leaves of Lophira lanceolate (Ochanaceae). *Life Science Journal*, 4(4):75–79.

Tiwari, P., Kumar, B., Kaur, M., Kaur, G., Kaur, H. 2011. Phytochemical screening and Extraction: A Review. *Internationale Pharmaceutica Scientia*, 1(1):98–106.

Winkel-Shirley, B. 2002. Biosynthesis of flavonoids and effects of stress. *Current Opinion in Plant Biology*, 5(3):218–223.

Woisky, R. G., Salatino, A. 1998. Analysis of propolis: some parameters and procedures for chemical quality control. *Journal of Apicultural Research*, 37(2):99–105.