ORIGINAL ARTICLE

INTERNATIONAL JOURNAL OF RESEARCH IN PHARMACEUTICAL SCIENCES

Published by JK Welfare & Pharmascope Foundation Journal Home Page: https://ijrps.com

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

Kousalya P[*1,2], Doss VA[1]

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

Article History:

Received on: 18 Sep 2020
Revised on: 10 Oct 2020
Accepted on: 19 Oct 2020

Keywords:

Artabotrys hexapetalus,
Primary metabolites,
Secondary metabolites,
Flavonoids,
Phenols,
Phytochemicals,
Tannins

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.

*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

IJRPS | https://ijrps.com

INTRODUCTION

Plants contain natural bioactive compounds called phytochemicals. Phytochemicals are classified into two categories primary metabolites and secondary metabolites (Krishnaiah *et al.*, 2009). Carbohydrates, amino acids, chlorophyll and proteins are primary metabolites. Secondary metabolites include alkaloids, flavonoids, terpenoids, tannins, phenols, glycosides, saponins (Krishnaiah *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.

Artabotrys hexapetalus (L.f.) Bhandari is a member of the Annonaceae family. It is located in India, South China and Srilanka. Within India, it

Table 1: 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)

is indigenous to south India and very commonly cultivated in gardens for its very fragrant flowers. *A.hexpetalus* 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 filterate 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

Artabotrys hexapetalus leaves extracted with aqueous (water), ethanol (alcohol), acetone, chloroform

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

and petroleum ether were analysed for qualitative 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 analysed by Folin-Denis method (Mohan, 2017).

Analysis of total phenols

Assessment of phenols in *Artabotrys hexapetalus* leaves was done by standard procedure (Malik and Singh, 1980).

Statistical analysis

Quantitative estimation of primary and secondary metabolites were done in triplicates with standards. The results of the quantitative analysis were given as mean ± standard deviation.

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).

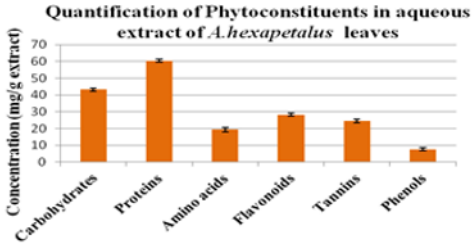

Figure 1: Quantification of Phytoconstituents in aqueous extract of *A.hexapetalus* leaves

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).

Analysis of tannins

The tannin content of aqueous extract of *A.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 Wallsgrove, 1994).

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. Yao Xue Xue bao=. *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 pinnatum (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., Sánchez, 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 Sciencia*, 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.