



CODEN [USA]: IAJPBB

ISSN: 2349-7750

**INDO AMERICAN JOURNAL OF  
PHARMACEUTICAL SCIENCES**<http://doi.org/10.5281/zenodo.1036003>Available online at: <http://www.iajps.com>

Research Article

**QUALITATIVE AND QUANTITATIVE ANALYSIS OF  
PHENOLS AND FLAVONOIDS IN FOLIAR EXTRACT OF  
ANTHOCEPHALUS CADAMBA (ROXB.).**Dr. Garima Bartariya<sup>1</sup>, Basant Kumar<sup>2</sup> and Abhishek Kumar<sup>3\*</sup><sup>1</sup>Department of Life Science, Itm University, Gwalior Madhya Pradesh  
E-mail- drgarimabartariya@gmail.com<sup>2</sup>Department of Biotechnology, Itm University, Gwalior Madhya Pradesh.  
E-mail- basantsingh31071994@gmail.com<sup>3</sup>Department of Biotechnology, Itm University, Gwalior Madhya Pradesh.  
E-mail- abhishekchaudhary964@gmail.com**Abstract:**

*Cadamba is an important medicinal plant used to treat various diseases like fever, anemia, diabetes and many others. The present study deals with the determination of total phenol and flavonoids content by using Spectroscopy. Anthocephalus cadamba is a medium sized plant which belongs to the family of Rubiaceae and found everywhere in India. This plant takes six to eight years for flowering and grows upto six meter in height. In the present study qualitative analysis was done to know the presence of various compounds where as the quantitative analysis was done to evaluate the concentration of them. Experiments were carried out for the phenol and flavanoids by using five solvents i.e petroleum ether, chloroform, ethanol, methanol and distilled water. Generally most of the phenolic compounds are linked to other compounds like proteins, cellulose, lignins and etc. Due to presence of these compounds phenols are important for the plant. Flavonoids are present in nature and have many biological activities such as anti-microbial, anti-inflammatory anti-tumor and many others. Result for phenol revealed that methanol extract contains higher phenol level. The concentration of phenol remains in between the range of 0.018128 - 0.559438 mgGA/g. It is lower in chloroform but higher in methanol. The order of the phenol content was chloroform < petroleum ether < distilled water < ethanol < methanol. Result for flavonoids shows that distilled water extract contains higher flavonoids content i.e 15.2 mg RU/g. The order of flavonoids content was distilled water> ethanol> methanol> chloroform> petroleum ether.*

**Keywords:** *Quantitative analysis, Qualitative analysis, Anthocephalus cadamba, Phenols, Flavonoids.***Corresponding author:****Abhishek Kumar,**

s/o- Arvind Kumar Chaudhay

At+PO- Ahiyari

Distt- Darbhanga, Bihar

PIN-847304

QR code



Please cite this article in press as Abhishek Kumar et al , *Qualitative and Quantitative Analysis of Phenols and Flavonoids in Foliar Extract of Anthocephalus Cadamba (ROXB.)*, Indo Am. J. P. Sci, 2017; 4(10).

**INTRODUCTION:**

*Anthocephalus Cadamba* (Roxb) is one of the important medicinal plants found in all parts of India. It is used in treatment of various disease like fever, anemia, diabetes, dysentery, upset stomach and wound healing. It also has antioxidant, antimalarial, antibacterial and antidiabetic activity. It is also known as wild cinchona in English [1]. Cadamba is a medium sized plant belongs to Rubiaceae family mainly found at low levels in wet places. It takes 6-8 years for flowering and grows upto 45 cm in height. The trunk has diameter of 100-160 cm. Leaves are 13-32 cm long, glossy green, opposite and simple. They are more or less sessile to petiolate, oval to elliptical (15-50\*8-25 cm). Flowers are bisexual and funnel shaped, sweetly fragrant and red to orange in color. Seeds are trigonal and irregular shaped. Bark is grey, smooth in young trees and rough in old ones [2].

Phenolics in plants are mostly synthesized from phenylalanine via the action of phenylalanine ammonia lyase i.e PAL [3]. They are classified into phenolic acid, flavonoid polyphenolics (flavones, flavonones, xanthenes and catechin) and non flavonoid polyphenols [4]. Phenolics are largest found and most widely distributed phytochemical, which act as a defence provider, natural antioxidants, anti-inflammatory agent to the plants [3].

Flavonoids are polyphenolic compounds, frequently found in nature, which occurs in vegetables, fruits, tea, coffee and fruit drinks [5]. They have many biological and pharmacological activities as antimicrobial, cytotoxicity, anti-inflammatory as well as anti-tumor activities [6]. They are made of more than one benzene ring in its structure. The compounds are derived from parent compounds known as flavons. Over 4000 flavonoids are known to exist and some are quercetin, kemferol and quercitrin [4].

**MATERIALS AND METHODS:**

The experiments were conducted in January- July 2017 in ITM University Gwalior Madhya Pradesh. Fresh and disease free leaves of *Anthocephalus cadamba* were collected from the botanical garden and used for solvent extraction. Collected leaves were washed with distilled water and shed dried at room temperature. The shed dried leaves were ground and stored in air-tight container for the further use of extraction with solvents. Five different solvents such as petroleum ether, chloroform, ethanol, methanol and distilled water were used for the extraction using soxhlet method.

**Qualitative Analysis:**

Qualitative analysis of phenol was done by following the methodology of Kalakotla et al. 2014, Sarojini et al. 2011 and Jayaramu et al. 2016 [7,8,9] for this two millilitre of test extract was

treated with 2 ml of 5% ferric chloride solution. Formation of blue colour indicated the presence of phenol.

**Flavonoids:**

For flavonoids alkaline reagent test was performed [7,8,9]. Few drops of sodium hydroxides solution was added in 2 ml of test extract. Intence yellow colour formed which turned into colour less solution on addition of few drops of dilute acid ( $H_2SO_4$ ). This change indicated that extract posses flavonoids in it.

**Quantitative Analysis:**

Quantitative estimation was done by using the method of Senguttuvan et al. 2014 and Jaradat et al. 2015 [10, 11].

**Flavanoids:**

The total flavonoids content was determined by using standard method of Senguttuvan et al. 2014 and Jaradat et al. 2015. For testing 0.1 ml of sample extract were mixed with 0.3 ml of distilled water followed by addition of 0.03 ml of 5% sodium nitrate ( $NaNO_2$ ). This mixture was incubated for 5 minutes at room temperature and 0.03 ml of 10% almunium chloride ( $AlCl_3$ ). After addition of almunium chloride it is again incubated for 5 minutes at room temperature followed by addition of 0.2 ml of 0.1 M sodium chloride ( $NaCl$ ). Absorbance was measured at 510 nm by spectroscopy (PerkinElmer). Distilled water was used as reference. Rutin were used as the standard compound.

**Phenols:**

Total concentration of phenol was determined by using standard method of Senguttuvan et al. 2014 and Jaradat et al. 2015. For testing 100 $\mu$ l of test sample was mixed with 105 ml of 5% Folin-Ciocalteu reagent and 1 ml of 20 % sodium carbonate ( $Na_2CO_3$ ). The mixture was shaken well and incubated for 20 minutes at room temperature. The absorbance was taken at 730nm by UV-Spectroscopy (PerkinElmer). Galic acid was used as the standard compound.

**RESULTS AND DISCUSSION:****Qualitative Analysis:**

Phenolics are largest found and most widely distributed phytochemical. It is found in foliar extract of methanolic, ethanolic as well as chloroform solvents. This result was supported by Jeyalalitha et al.2015 [12].

Flavonoids are polyphenolic compounds, made up of more than one benzene ring and found only in distilled water. The findings of Padalkar et al.2013 [13] and Acharyya et al.2010 [14] also show the presence of flavonoid in distilled water extracts which enhance its importance in pharmacological field [13, 14].

**Table 1: Quantitative screening of Phenol and Flavonoids in different solvent extract in leaves of *Anthocephalus cadamba*.**

Phytoconstituents	Petroleum ether	Chloroform	Ethanol	Methanol	Distilled water
Phenol	-	+	+	+	-
Flavonoids	-	-	-	-	+

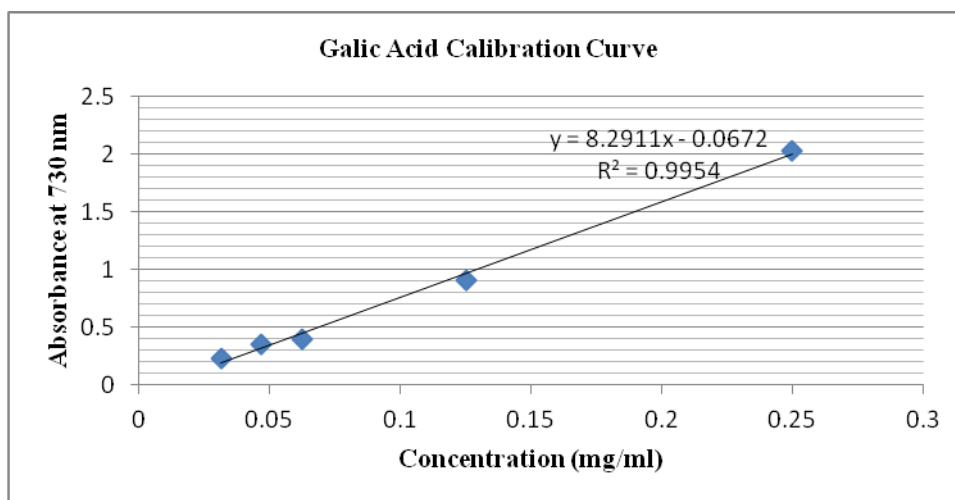
**Quantitative Analysis:****PHENOLS:**

Result of this study revealed that methanol extract contains higher phenol level. The concentration of phenol remains in between the range of 0.018128 - 0.559438 mgGA/g. It is lower in chloroform but higher in methanol. The order of the phenol content was chloroform < petroleum ether < distilled water < ethanol < methanol i.e 0.018128, 0.09532, 0.15635, 0.32364, 0.559438 respectively. Methanol is a good solvent for the extraction of phenol and

this result was also supported by Ghatak et al. 2015 and Formagio et al. 2014 [15, 16]. The phenolic compounds are essential for the plant tissues because it plays vital roles in plants such as nutrient uptake, photosynthesis, protein synthesis and many others [15, 17]. Generally most of the phenolic compounds are linked to other compounds like proteins, esters, cellulose and lignins [15, 18]. Therefore they are very important compounds because their hydroxyl groups confer scavenging ability [15].

**Table 2: Absorbance of Standard Compound (Gallic acid) at  $\lambda = 730\text{nm}$** 

Gallic acid concentration (mg/ml)	Absorbance (mean) at 730nm
0.0312	0.236
0.0468	0.356
0.0625	0.401
0.125	0.909
0.25	2.036

**Fig 1: Standard Calibration Curve of Gallic acid****Table 3: Total Phenolic Content in Different Extract of *Anthocephalus cadamba*.**

S.no	Solvents	Total Phenolic content (mg GA/g) $\pm$ SD
1	Petroleum ether	0.09532 $\pm$ 0.006
2	Chloroform	0.018128 $\pm$ 0.001
3	Methanol	0.559438 $\pm$ 0.14
4	Ethanol	0.32364 $\pm$ 0.09
5	Distilled water	0.15635 $\pm$ 0.06

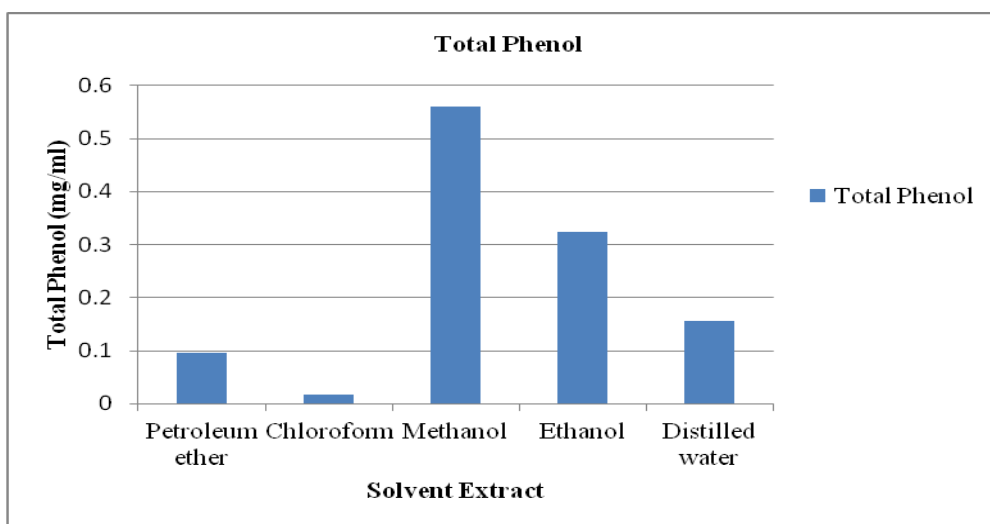


Fig 2: Total Phenol Content in Different Extract of *Anthocephalus cadamba*

#### FLAVONOIDS:

Flavonoids occur naturally occurring phytochemical of the plant and possess antibacterial, antiviral, anti-inflammatory, anticancer and anti-allergic activities [15, 19]. The total flavonoids content was determined by the following standard method of Senguttuvan et al. 2014 [10]. Result of this study shows that distilled water extract contains higher flavonoids content in it which is similar to the result of Ghatak et al. 2015 [15]. The order of flavonoids content was distilled water >

ethanol > methanol > chloroform > petroleum [15]. Study of Senguttuvan et al. 2014 [10] reported that the flavonoids content was absent in petroleum ether and chloroform whereas study of Ghatak et al. 2015 [15] reported that the ethanolic extract of leaf and methanolic extract of bark contained highest flavonoids content. Flavonoids and phenols are however reported in present study which agrees with the findings of Senguttuvan et al. 2014 and Ghatak et al. 2015 [10, 15].

Table 4: Absorbance of Standard Compound (Rutin) at  $\lambda = 510 \text{ nm}$

Rutin concentration ( $\mu\text{g/ml}$ )	Absorbance (mean) at 510nm
0.3	0.126
0.6	0.176
1.2	0.201
2.4	0.368
6	0.588

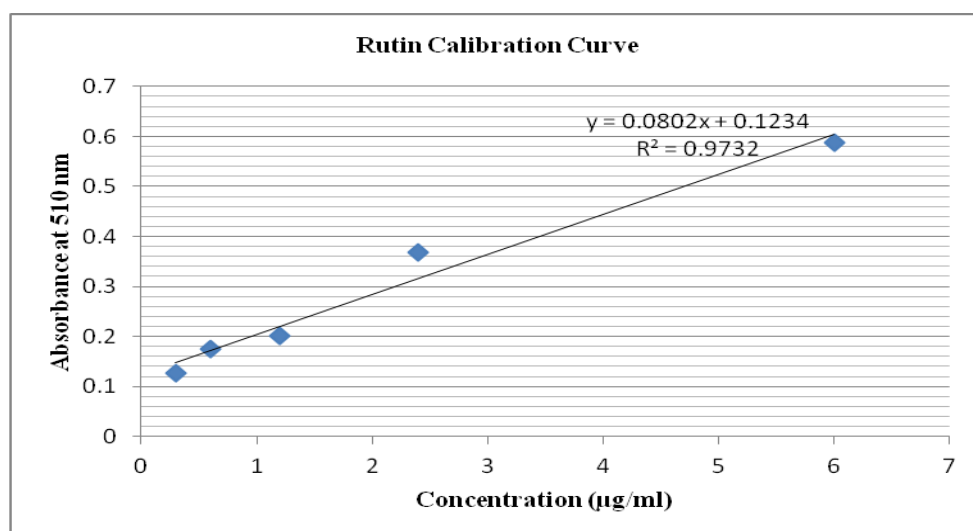
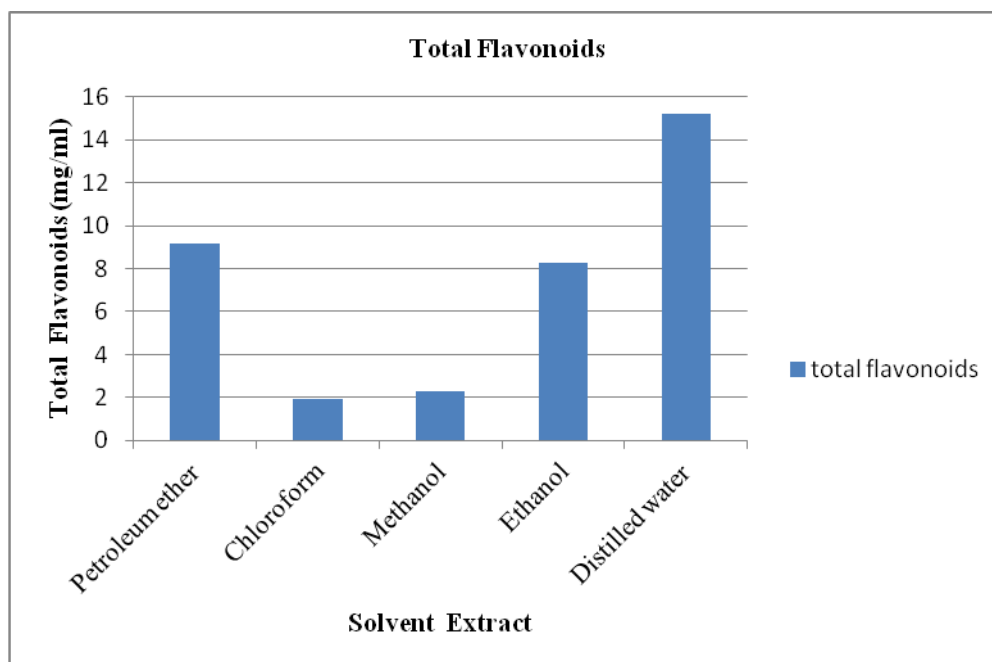


Fig 3: Standard Calibration Curve of Rutin

**Table 5: Total Flavonoids Content in Different Extract of *Anthocephalus cadamba*.**

S.no	Solvents	Total Flavonoid content (mg RU/g) $\pm$ SD
1	Petroleum ether	9.187 $\pm$ 2.21
2	Chloroform	1.95 $\pm$ 0.57
3	Methanol	2.275 $\pm$ 1.36
4	Ethanol	8.25 $\pm$ 8.25
5	Distilled water	15.2 $\pm$ 4.46

**Fig 4: Total Flavonoids Content in Different Extract of *Anthocephalus cadamba*****CONCLUSION:**

Research on medicinal plant is the subject of great interest recently, which is the reason to explore the medicinal importance of this plant. Qualitative analysis for phenols and flavonoids were performed in the foliar extract of *Anthocephalus cadamba* in five different solvent. Present study revealed the presence of phenols in chloroform, ethanol and methanol whereas flavonoids was present in only distilled water. The quantitative study revealed that order of concentration of phenols was chloroform < petroleum ether < distilled water < ethanol < methanol whereas for flavonoids it was distilled water > ethanol > methanol > chloroform > petroleum ether. Quantitative screening of phenols and flavonoids were performed by using UV spectrometer for more accuracy of results.

**REFERENCES:**

1. Padalkar, S., Palshikar, G. Firake, B. and Parekh, P. Pharmacognostic evaluation and phytochemical screening of *Anthocephalus cadamba*. Asian Journal of Research in Biological and Pharmaceutical Sciences. 2013; 1 (2): 86-96.

2. Dwevedi, A., Sharma, K. and Sharma, Y.K. *Cadamba*: A miraculous tree having enormous pharmacological implications. Pharmacognosy Review. 2015; 9 (18): 107-113.

3. Gryglewski, R.J., Korbut, R. and Robak, J. On the Mechanism of Antithrombotic Action of flavonoids. Biochemical Pharmacology. 1987; 36: 317-321.

4. Saxena, M., Saxena, J., Nema, R., Singh, D. and Gupta, A. Phytochemistry of medicinal plants. Journal of Pharmacognosy and Phytochemistry. 2013; 1(6): 168-182.

5. Pridham, J.B. In: phenolic in plants in health and disease. Pergamon Press, New York. 1960; 34-35.

6. Pretorius, J.C. Flavonoids: A review of its commercial application potential as anti-infective agents. Current Medicinal Chemistry- Anti-Infective Agents. 2003; 2: 335-353.

7. Kalakotla, S., Gottumukkala, K.M., Rani, M.S., Divya, L. and Pravallika, P.L. Screening of *Saraca indica* (linn.) medicinal plant for antidiabetic and antioxidant activity. Der Pharmacia Lettre. 2014; 6(4): 227-233.

8. Sarojini, N., Sahoo, A. M. and Chakraborti, C.K. Phytochemical and anthelmintic activity study of

- Saraca indica* leaves extracts. International Research Journal of Pharmacy. 2011;2(5): 194-197.
- 9.Prabhavathi,R.M., Prasad, M.P. and Jayaramu M. Studies on qualitative and quantitative phytochemical analysis of *Cissus quadrangularis*. Advance in Applied Science Research. 2016;6(7): 11-17.
- 10.Senguttuvan, J., Paulsamy, S. and Karthika, K. Phytochemical analysis and evaluation of leaf and root parts of the medicinal herb, *Hypochaeris radicata* L. for in vitro antioxidant activities. Asian Pacific Journal of Biomedicine. 2014;4(1): S359-S367.
- 11.Jaradat, N., Hussien, F. and Ali, A.A. Preliminary phytochemical screening, quantitative estimation of total flavonoids, total phenols and antioxidant activity of *Ephedra alata* decne. Journal of Material and Environmental Science. 2015;6 (6): 1771-1778.
- 12.Jayalalitha, T. Murugan, K and Umayavallin, M. Preliminary phytochemical screening of leaf extracts of *Anthocephalus cadamba*. Journal of Recent Scientific Research.2015; 10(6): 6608-6611.
- 13.Padalkar, S., Palshikar, G. Firake, B. and Parekh, P. Pharmacognostic evaluation and phytochemical screening of *Anthocephalus cadamba*. Asian Journal of Research in Biological and Pharmaceutical Sciences.2013; 1 (2): 86-96.
- 14.Acharyya, S., Dash, G.K., Mondal, S. and Dash, S.K. Studies on glucose lowering efficacy of the *Anthocephalus cadamba* roots. International Journal of Pharma and Bio Science.2010; 1 (2): 2-9.
- 15.Ghatak, A., Nair, S., Vajpayee, A., Chaturvedi, P., Samant, S., Soley,K., Kudale, S. and Desia, N. Evaluation of antioxidant activity, total phenolic content, total flavonoids and LC-MS characterization of *Saraca asoca* (Roxb.) De. Wild. International Journal of Advanced Research.2015; 3(5): 318-327.
- 16.Formagio, A.S.N., Volobuff, C.R.F., Santiago, M., Cardoso, C.A.L., Vieira, M.C., Pereira, Z.V. Evaluation of antioxidant activity, total flavonoids, tannins and phenolic compounds in psychotria leaf extract. Antioxidant. 2014;3: 745-757.
- 17.Goleniowski, M., Bonfill, M., Cusido, R., Palazo'n, J Phenolic acid. Journal of Natural Product. 2010;63 (1): 1951-1973.
- 18.Sadhu, S.K., Khatun, A., Phattanawasin, P., Ohtsuki, T., Ishibashi, M. Lignin glycosides and flavonoids from *Saraca asoca* with antioxidant activity. Journal of Natural Medicine.2007; 61: 480-482.
- 19.Saeed, N., Khan, M.R., Shabbir, M. Antioxidant, total phenolic and flavonoi content of whole plant extract *Torilis leptophylla*. BMC Complementry and Alternative Medicine. 2012;12: 1-12.