WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 7.523
Volume 6, Issue 4, 1066-1075. Research Article ISSN 2277– 7105

PHYTOCHEMICAL ANALYSIS AND SCREENING OF TOTAL FLAVONOID, TANNIN AND PHENOLIC CONTENTS IN *CROTON BONPLANDIANUM* LEAF AND STEM

Dr. A. Chandra Mohan*[1] and Dr. S. R. Divya[2]

*[1]Associate Professor, [2]Assistant Professor, Department of Biochemistry and Chemistry, Jaya College of Arts and Science, Thiruninrayur, Chennai, Tamilnadu.

Article Received on
23 Jan. 2017,

Revised on 13 Feb. 2017,
Accepted on 07 March. 2017

DOI: 10.20959/wjpr20174-8142

***Corresponding Author**
Dr. A. Chandra Mohan
Associate Professor,
Department of Biochemistry and Chemistry, Jaya College of Arts and Science,
Thiruninrayur, Chennai,
Tamilnadu.

ABSTRACT

The aim is to study the phytochemical constituents present in *Croton bonplandianum* leaf and stem. Preliminary phytochemical analysis revealed the presences of phytochemicals such as carbohydrates, quinines and glycosides in all the tested extracts in both the leaf and stem of *Croton bonplandianum*. Coumarins, proteins, steroids, phytosteroids, phlobatannins and anthraquinones were absent in all the tested extracts. The amount of total phenolics, total flavonoids and tannin content in hexane, ethyl acetate and methanol extracts of leaf and stem were determined spectrometrically. The hexane, ethyl acetate and methanol extract of stem showed higher total phenolic, flavonoid and tannin content then leaf. The present study suggested that, detailed studies on the isolation and characterization of the leaf and stem extracts as well as investigations on other biological studies and in vivo assays will be interesting in discovering new drugs.

KEYWORDS: *Croton bonplandianum*, phytochemical screening, medicinal uses and TPF.

INTRODUCTION

Traditional folk medicine involves use of herbal and natural products to treat various diseases. Reports from WHO states that 70- 80% of the total population in the world use herbs as alternative medicine (Divya *et al*.,2011). In India, over 7500 plant species are being used in traditional medicines (Singh *et al*., 2011). Euphorbiaceae family in the plant kingdom is a complex hetero-geneous family consisting of about 322 genera and 8900 species in the

world. *Croton bonplandianum* Baill belonging to Euphorbiaceae famlily is a native of southern Bolivia, Paraguay, Southwestern Brazil, and Northern Argentina (Chakrabarty and Balakrishnan, 1992). In India, *C.bonplandianum*, commonly called as "Railpachilai" in South and "Ban Tulsi" in North are found abundant in Malda, West Bengal and Assam (Mahadeswara Swamy, 2006). Its stem and bark paste is used for treating skin diseases, treat headaches and to arrest bleeding wounds (Balasubramanian *et al*., 1997, Ajit *et al*., 2007, J. Lenin and Venkat., 2009). Stem and branches are used as fuel and its ashes are used as detergents in interior eastern India (Mahadeswara Swamy, 2006). On the other hand, seeds are used for the treatment of jaundice, acute constipation abdominal dropsy and internal abscesses (Divya *et al*., 2011).

MATERIALS AND METHODS

Plant collection

Healthy, disease free plant of *Croton bonplandianum* were collected from Redhills, Chennai. Collected plant was authenticated by Plant Anatomy Research Centre (PARC/2012/1307). Leaves were washed and air dried. They were then powdered using liquid nitrogen and used for further assay.

Estimation of Total phenolic, flavonoid and tannins content

Reagents required

- Folin-Ciocalteu reagent
- SAodium carbonate -75g/L
- gallic acid (1mg/ml)
- Leaf extracts (1mg/ml)
- Quercitin (1mg/ml)
- 5% sodium nitrite solution
- 10% aluminium chloride
- 1M sodium hydroxide
- Folin–Denis reagent
- 15% sodium carbonate
- Tannic acid (1mg/ml)
- Leaf extracts (1mg/ml)

Phytochemical screening

Plant materials were collected and processed for a sequential cold percolation method using Hexane, Diethyl ether, Ethyl acetate, and methanol. The phytochemical analysis of *Croton bonplandianum* leaf and stem were done to detect the presence of carbohydrates, tannins, saponins, flavonoids, alkaloids, quinones, glycosides, terpenoids, triterpenoids, phenols, coumarins, steroids and phytosteroids, phlobatannins and anthraquinones indicated results as given in the (Table 1 and Table 2) respectively.

Table 1: Phytochemical analysis of leaf extracts

Test	Hexane	Diethyl ether	Ethyl acetate	Methanol
Carbohydrates	+	+	++	+
Tannins test	+	-	+	+
Saponin test	-	-	-	+
Flavonoid test	+	-	+	+
Alkaloid test	-	-	+	+
Quinones	-	-	-	-
Glycosides test	-	-	-	-
Cardiac glycosides test	-	-	+	+
Terpenoids test	-	-	-	-
Triterpenoids	-	-	-	-
Phenols	+	+	+	+
Coumarins	-	-	-	+
Steroids and Phytosteroids	-	-	-	+, -
Phlobatannins	-	-	-	-
Anthraquinones	-	-	-	-

'+' indicates presence and '-' indicates absence

Table 2: Phytochemical analysis of stem extracts

Test	Hexane	Diethyl ether	Ethyl acetate	Methanol
Carbohydrates	+	+	+	+
Tannins test	+	-	+	+
Saponin test	+	+	-	+
Flavonoid test	+	-	+	+
Alkaloid test	+	+	+	-
Quinones	-	-	-	-
Glycosides test	-	-	-	-
Cardiac glycosides test	+	-	-	-
Terpenoids test	-	-	-	-
Triterpenoids	-	-	-	-
Phenols	+	+	+	+
Coumarins	+	-	-	+
Steroids and Phytosteroids	+, -	-	+, -	-
Phlobatannins	-	-	-	-
Anthraquinones	-	-	-	-

'+' indicates presence and '-' indicates absence

Estimation of Total phenolic content

Total Phenolic content (TPC) in the leaf and stem extracts (Ethyl acetate and methanol) was determined using the Folin-Ciocalteu reagent method (Miliauskasa, G, *et al.,* 2004). This method depends on the reduction of FCR by phenols to a mixture of blue oxides which have a maximal absorption in the region of 765 nm using spectrophotometer. Stock solution of leaf extracts was prepared to the concentration of 1mg/ml. To 0.1ml of each extract, 5ml of Folin-Ciocalteu Reagent were added. The mixture solution was vortexed and incubated in the dark for 3 minutes, respectively. To the incubated content 5 ml of sodium carbonate (75g/L) solution was added to the above content and mixed thoroughly. The reaction content was incubated in the dark for 1 hour. The absorbance was read at 765 nm. Blank was maintained with 5 ml Folin-Ciocalteu reagent, 1 ml ethanol and 4 ml sodium carbonate solution. The concentration of total phenolic content in the extract was expressed as mg of gallic acid equivalent per g of dry weight (mg GAE/g) of extract. Gallic acid stock solution was prepared to the concentration of 1 mg/ml. Serial dilution was carried out; gallic acid solution was dissolved in ethanol. A linear dose- response regression curve was generated using absorbance reading of gallic acid at the wavelength of 765 nm.

Estimation of Total flavonoid content

Total flavonoid content (TFC) in the leaf and stem extracts (ethyl acetate and methanol extract) was determined using the method described by (Sakanaka, S, *et al.*, 2005). The flavonoid content was determined by aluminium chloride method using Quercitin as standard. Extracts and Quercitin were prepared in ethanol (1 mg/ml). 0.1ml of extract was mixed with 0.9ml of distilled water in test tubes, followed by addition of 75 µL of a 5% sodium nitrite solution. After six minutes, 150µL of a 10% aluminium chloride solution was added and the mixture was allowed to stand for further five minutes after which 0.5 ml of 1M sodium hydroxide was added to the reaction mixture. The reaction mixture was brought to 2.5 ml with distilled water and mixed well. The absorbance was measured immediately at 510 nm using a spectrophotometer. Determination was performed in three replicates. A calibration curve was generated using various concentrations of Quercitin (20 - 140µg). Blank consist of all the reagents, except for the extract or Quercitin standard solution is substituted with 0.1 ml of ethanol. Results were expressed as mg of Quercitin equivalent/g of dry weight (mg QE/g) of extracts.

Estimation of total tannins

Total Tannin content in the leaf extracts (ethyl acetate and methanol extract) was determined by Folin–Denis method (Schanderi 1970) with minor modifications. Stock solution of leaf extracts was prepared to the concentration of 1mg/ml. To 0.1ml of each extract, 1ml of distilled water was added and then mixed with 0.5 ml of Folin–Denis reagent. The reaction mixture was alkalinized by the addition of 1 ml of 15% (w/v) sodium carbonate solution and kept in dark for 30 min at room temperature. The absorbance of the solution was read at 700 nm using spectrophotometer, and the concentration of tannin in the extract was determined using pure tannic acid as standard (1mg/ml). A calibration curve was generated using various concentrations of Tannic acid (20 - 120µg) was obtained. Blank consist of all the reagents, except for the extract or standard solution is substituted with 0.1 ml of water. Results were expressed as mg of Tannic acid equivalent/g of dry weight (mg TE/g) of extracts.

RESULT AND DISCUSSION

The preliminary phytochemical analysis of Hexane, Ethyl acetate and Methanol extracts of *Croton bonplandianum* leaf samples shows the strong presence of Carbohydrates, Tannins, Flavonoids and Phenols in all the three extracts, except Diethyl ether showed Carbohydrates and Phenols. There is a sparse presence of alkaloids and glycosides in the ethyl acetate and methanol extracts. Saponin, coumarins and steroids are present in the methanol extract. The phytocompounds quinones, glycosides, terpenoids, triterpenopids, phlobatannins and anthraquinones are absent in all the four extracts. Similarly the preliminary phytochemical analysis of the stem samples shows the strong presence of carbohydrates, tannins, flavonoids and phenols. Alkoloids and steroids are present in the hexane and ethyl acetate samples. Saponins are present in the hexane and methanol samples. The other phytochemicals namely quinones, glycosides, terpenoids, triterpenopids, phlobatannins and anthraquinones are absent in stem and leaf. Based on the presence of phytocompounds the further estimation will carried out with three solvent Hexane, Ethyl acetate, and methanol.

As phytochemicals often play an important role in plant defense against prey, microorganism, stress as well as interspecies protections, these plant components have been used as drugs for millennia and hence, screening of phytochemicals serves as the initial step in predicting the types of potential active compounds from plants (Chew *et al*, 2011).

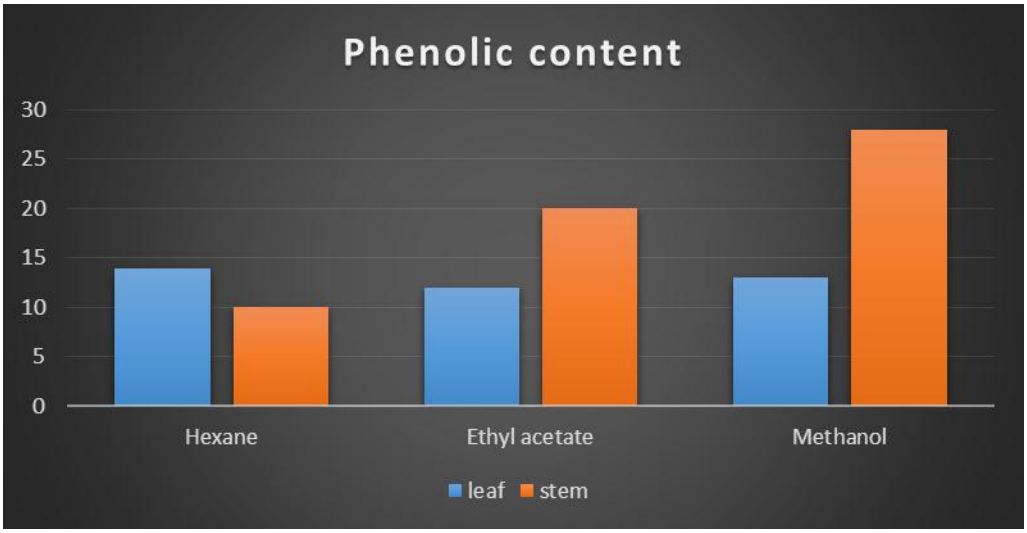
Flavonoid compounds especially quercetin and genistein have antitumor activity and these compounds are cytotoxic to cancer cells but have no or insignificant activity in normal cells

(Pouget *et al*, 2001). It has been reported that flavonoid, apigenin holds great promise as a chemopreventive agent for a variety of cancers and exhibits significant activity against UV induced DNA damage and thus protect against skin cancer (Baliga and Katiyar, 2006). Plant phenolics are a major group of compounds that act as primary antioxidants of free radical scavengers (Polterait, 1997).

These compounds present in a variety of medicinal plants have significant application against human. Pathogens, including those that cause enteric infections and are reported to have curative properties against several pathogens and therefore could suggest their use in the treatment of various diseases (Hassan *et al*., 2004). Alkaloids are formed as metabolic byproducts and have been reported to be responsible for the antibacterial activity in most of the medicinal plants (Mantle *et al*, 2000). Glycosides serve as defence mechanisms against predation by many microorganisms, insects and herbivores (Dhar *et al*, 1979).

Estimation of Total phenolic content (TPC)

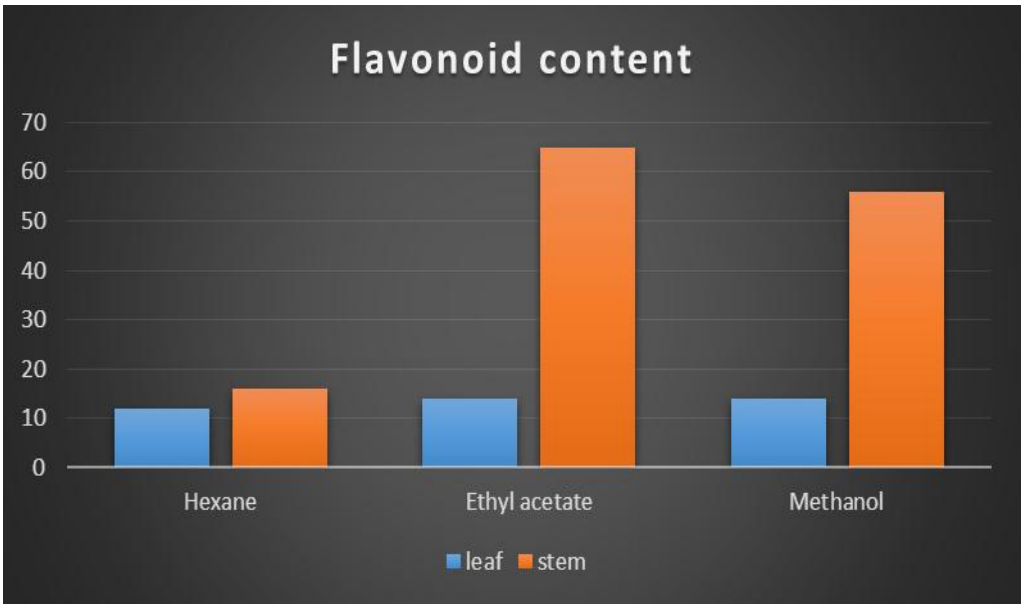
The concentrations of total phenolic content in the extracts were expressed as mg of gallic acid equivalent per g of dry weight (mg GAE/g) of extract the total phenolic content of the leaf and stem samples of *Croton bonplandianum* were determined using the Folin-Ciocalteu reagent method. The reduction of FCR by phenols to a mixture of blue oxides which have a maximal absorption in the region of 765 nm was measured spectrophotometrically. The results revealed the presence of highest total phenol content in the hexane extract of the leaf in *C. bonplandianum* is 14µg/g whereas the total phenol content in the methanol extract of the stem is 28µg/g. The methanol and ethyl acetate leaf extracts contain the total phenol of 13µg/g and 12µg/g respectively. The hexane and ethyl acetate stem extracts contain 10µg/g and 20µg/g of total phenol content (“Fig. 1”). Phenolic compounds possess different biological activities, but most important are antioxidant activities. Phenols are able to scavenge reactive oxygen species due to their electron donating properties (Re *et al*., 1999 and Velioglu *et al*., 1998).



"Fig. 1", Estimation of total phenolic content from leaf and stem extracts

Estimation of Total flavonoid content

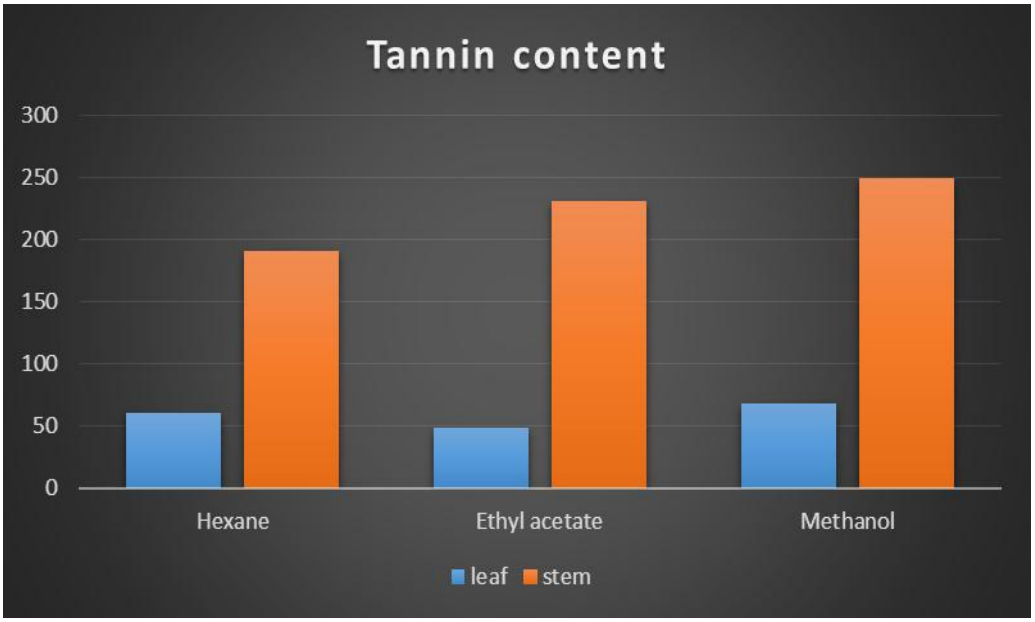
Total flavonoid content of samples was obtained in comparison with the Quercitin standard. The total flavonoid content of the leaf and stem samples of *Croton bonplandianum* were determined by aluminium chloride method. The results made known the presence of highest flavonoid content in the ethyl acetate and methanol extracts of the leaf to be 14µg/g, whereas the ethyl acetate extract of the stem contains the highest flavonoid content of 65µg/g. The hexane extract of the leaf contains 12µg/g, the hexane and methanol extract of the stem contains 16µg/g and 56µg/g of total flavonoid. The results indicate that the stem of *Croton bonplandianum* contains more flavonoids than the leaf ("Fig. 2").



"Fig. 2", Estimation of total flavonoid content from leaf and stem extracts

Estimation of total Tannin

The total tannin content was expressed as mg of Tannic acid equivalent/g of dry weight (mg E/g) of extracts. The total Tannin content of the leaf and stem samples of *Croton bonplandianum* were determined using leaf extracts measured by Folin–Denis method. The results specify that the total tannin content in the methanol extract of the leaf is 68µg/g, whereas the ethyl acetate and the methanol extracts contain 60µg/g and 48µg/g of tannin content. Comparatively the methanol extract of the stem contains 249µg/g, the hexane and methanol extracts contain 91µg/g and 231µg/g of tannin respectively. The results designate that the stem of *Croton bonplandianum* contain more tannin when compared to the leaf ("Fig. 3").



"Fig. 3", Estimation of total tannin content from leaf and stem extracts

CONCLUSION

The present study of the phytochemical screening, total phenolics, total flavonoids and total tannins of different extracts in *Croton bonplandianum* leaf and stem showed that, these plant could be a potential source for natural antioxidants. It has been reported that most active principles in *Croton bonplandianum* are frequently alkaloids, flavonoids and phenols and these may be responsible for many of the pharmacological actions of the particular plant. If these plants are examined for further biological studies, it could be a promising agent in scavenging free radicals and treating diseases related to free radical reactions. Furthermore, detailed studies on the isolation and characterization of the plant extract as well as studies

with other models such as lipid peroxidation and *in vivo* assays will be interesting in discovering new drugs.

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