Int J Pharm Bio Sci 2013 Apr; 4(2): (P) 966 - 972

Research Article **Medicinal Chemistry**

International Journal of Pharma and Bio Sciences **ISSN 0975-6299**

ISOLATION AND IDENTIFICATION OF GALLIC ACID FROM *POLYALTHIA LONGIFOLIA* (SONN.) THAWAITES

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ABSTRACT

Isolation and purification of bioactive compounds from naturally occurring substance will play a central role in the development and modernization of phytomedicine.The study was carried out to the isolate and simultaneous identification of gallic acid from the Ethanolic Extract of *Polyalthia Longifolia* (Sonn.) Thawaites leaves (EEPL). The simple, rapid and cost effective Preparative Thin Layer Chromatography (PTLC) was used for the identification and isolation of gallic acid. R_f value of PTLC isolate and reference standard gallic acid was found to be 0.47 and 0.48 respectively. Reverse Phase High Performance Liquid Chromatography (RP-HPLC) fingerprint of PTLC isolate of EEPL also revealed that the presence of gallic acid.

KEYWORDS: ***Polyalthia longifolia*, Phytomedicine, Phenolic acid, PTLC isolate, Antioxidant.**

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1. INTRODUCTION

Phenolic acids have been considered as potential therapeutic agents against a wide range of ailments including neurodegenerative diseases, cancer, diabetes, cardiovascular dysfunction, inflammatory diseases and in ageing[1]. They are widely distributed in the plant kingdom, therefore an integral part of the diet with significant amounts being reported in vegetables, fruits and beverages. They received particular attention in the past 10 years because of their putative role in the prevention of several human diseases, particularly diabetics and cancer. The importance of antioxidant activities of phenolic acids and their possible usage in processed foods as a natural antioxidant have reached a milestone both in food biotechnology and in the health care sector. Phenolic acids are diverse group that includes the widely distributed hydroxybenzoic and hydroxycinnamic acids[2]. Several phenolic acids[3] were reported (i.e Ferulic acid, ellagic acid, caffeic acid etc.,) from plants. One such prominent phenolic acid is gallic acid. It is found in a wide variety of foods including vegetables, fruits, tea, coffee, and wine. Gallic acid elicits several interesting and various biological responses, such as antibacterial, anti-fungal, anti-inflammatory, antiviral, anticancer, antioxidant[4], antimutagenic, and anti-diabetic activities. Due to these biological activities, gallic acid could be a good lead compound for new drug development. *Polyalthia Longifolia* (Sonn.) Thawaites (Annonaceae) is a tree (Figure 1), which is widely distributed in throughout the hotter parts of India[5]. A number of biologically[6] active compounds (alkaloid, flavonoids, saponins, sterols, terpenoids etc.,) have been isolated from this plant. In India, *Polyalthia longifolia* species were traditionally[7] used to treat the various diseases like fever, skin diseases, gonorrhea, analgesic, helminthiasis, hyperglycemic, hypertension, ulcer and cancer. Most of the plants of the Annonaceae family posses antimicrobial, antitumor, antioxidant potential.

Figure 1
Polyalthia Longifolia (Sonn.) Thawaites

Recently, leaves of *Polyalthia longifolia* extract and isolated compounds were studied for various biological activities[8] like antibacterial, anticancer and antioxidant. Further screening of

this medicinal plant may result in the discovery of novel effective compounds. A survey of literature revealed that no simple, rapid and cost effective methodical reports on isolation identification of gallic acid from ethanolic extract of *Polyalthia Longifolia.* In this connection, the current study is trying to explore a suitable and cost effective method for the isolation and identification of gallic acid from ethanolic extract of *Polyalthia Longifolia* (Sonn.) Thawaites leaves.

2. MATERIALS AND METHODS

2.1 Plant material and extraction process

The leaves were collected from the medicinal garden of Cymbio Pharma Pvt Ltd., Bangalore, India. The species was identified and confirmed at the Botanical Survey of India (BSI), Southern Circle, Coimbatore, India. The voucher specimen of the sample (SAM-06) was deposited at the Department of Research and Development, Cymbio Pharma Private Limited., Bangalore, Karnataka, India. About 10g of air-dried leaves were dissolved in 100ml of the ethanol and extracted at room temperature for 12 hours with occasional stirring[8]. The obtained extracts were filtered with Whatman No.1 filter paper. The ethanolic extract was concentrated under reduced pressure in a rotary evaporator. Finally, the gummy like residue was stored for further analysis at 4^0C.

2.2. Isolation of gallic acid by PTLC

PTLC was carried out for the identification and isolation of gallic acid present in EEPL. The PTLC of gallic acid was performed using solvent system[10] i.e Chloroform: ethanol: formic acid. Finally, PTLC plate was dried and sprayed with detecting reagent.

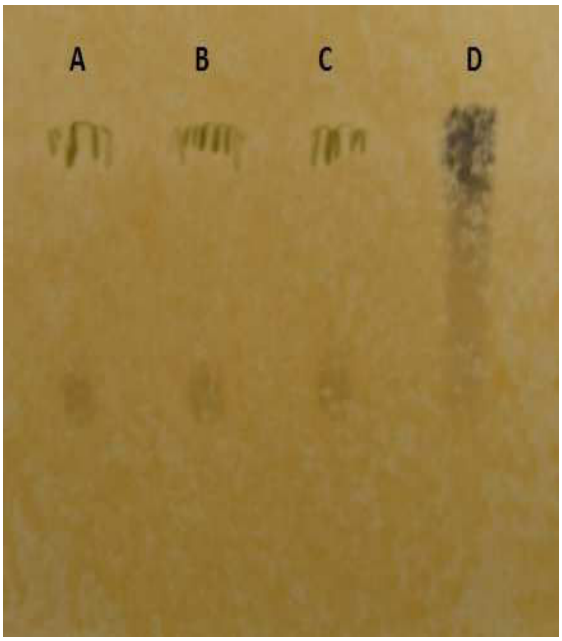
2.3 RP- HPLC analysis of gallic acid

HPLC separation was accomplished on an Agilent LC-9A model instrument. For analysis, a linear isocratic elution programme was applied, and elution was carried out with mobile phase (acetonitrile/methanol (50:50 v/v)). Flow rate was 1.0 mL/min and UV absorbance was set at 280 nm[11]. The mobile phase was filtered and degassed before use. The column was equilibrated with mobile phase. 50 µl of PTLC purified sample was filtered through membrane filter (0.22 µm) and then injected into the sample introduction system. The reference standard (gallic acid) was analyzed under the same conditions.

3. RESULT AND DISCUSSION

The phenolic acids exhibit a wide range of biological effects including antibacterial, anti-inflammatory, antiallergic, hepatoprotective, antithrombotic, antiviral, anticarcinogenic and vasodilatory actions. Recent years, gallic acid have quit interest of in food, cosmetic and pharmaceutical industries, as substitutes for synthetic antioxidants[12, 13]. Several HPLC methods have been documented for identification and isolation of gallic acid in plant extracts, but due to complex nature and inherent variability of the chemical constituents in the plant-based drugs, it is difficult to establish the simple and cost effective chromatographic technique for isolation and purification purpose. To overcome these problems in the current study, PTLC was used for the identification and isolation of gallic acid from ethanolic extract of *Polyalthia Longifolia* leaves. In recent years, TLC and PTLC has been successfully used for isolation and online purification of secondary metabolites[14] such as phenolic acids, flavonoids [15] and alkaloids from plant source. It has many advantages over the column chromatography (i.e. economical, rapid etc.,). The glass plate (10 x 5 cm) was coated with silica gel 'G' (0.5-1.0 mm thick) for the PTLC analysis. Generally, increase the thickness of adsorbent gives good yield. In addition, decrease the thickness of adsorbent results good resolution of particular analyte. Both semi qualitative and quantitative analysis of plant metabolites, uniform thickness of adsorbent only used in PTLC. The plate was activated at 100°C for 30 minutes in an oven and cooled at room temperature. The EEPL was spotted 1 cm above the edge of the plate

along with reference standard gallic acid. The glass plate was developed in TLC chamber containing about 300 ml of solvent mixture of chloroform, ethanol, formic acid in (85: 15: 1) ratio. The developed plate was air dried and visualized under UV light that showed fluorescent spots and coinciding with the reference standard. Recently, PTLC was used[16] for the isolation of quercetin from the leaves of *Citrullus Colocynthis* (Linn.) Schrad. Similarly, it has been used for identification and isolation of rutin[8] related flavonoids from ethanolic extract of *Polyalthia Longifolia* leaves. In addition, [17] has successfully isolated flavonoids and phenolic acid from the flowers of *Tabernaemontana heyneana*. The developed plates was sprayed[10] with 1% ferric chloride solution (Figure 2). R_f value was calculated for EEPL and compared with coinciding standard. Each of the fluorescent spots compared with those of reference standard was marked. The predicted strong spots were carefully scrapped and collected separately along with the silica gel and eluted with methanol by mild centrifugation[17]. Finally, the isolated material was subjected to HPLC studies.



A-C-EEPL**; D-**Gallic acid

Figure 2
Isolation of gallic acid from EEPL by PTLC and visualized after application of 1% ferric chloride

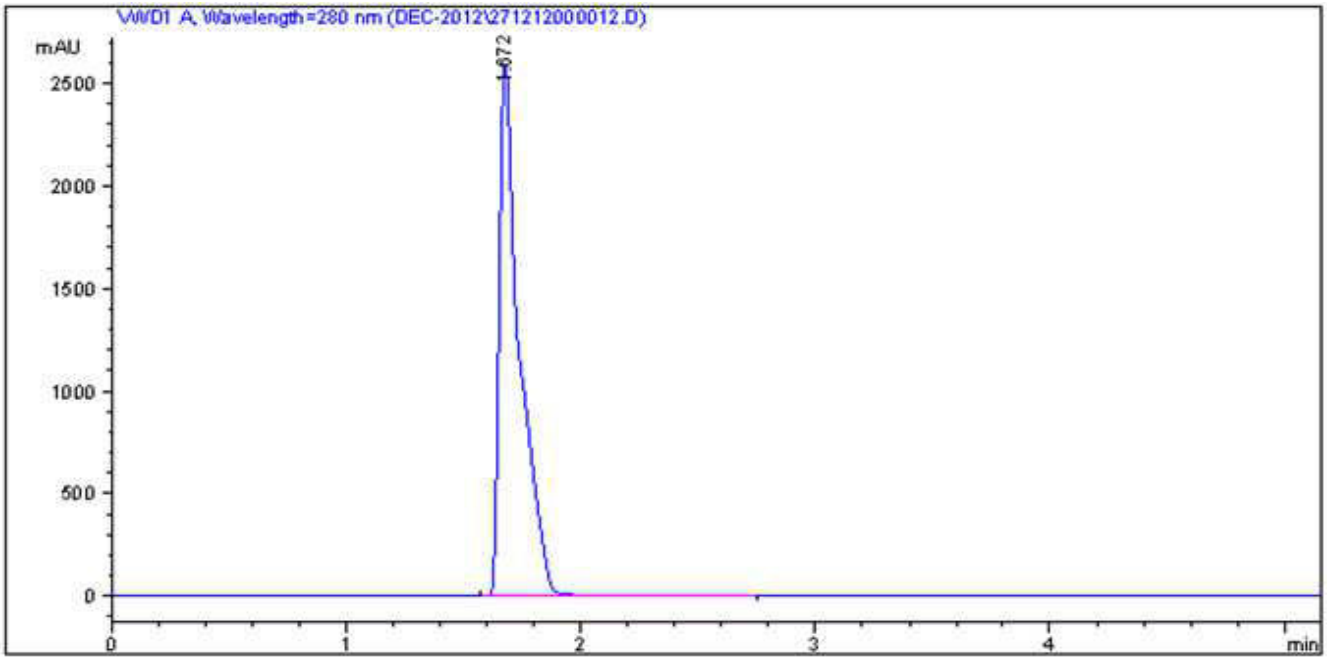


Figure 3
RP- HPLC spectrum of gallic acid

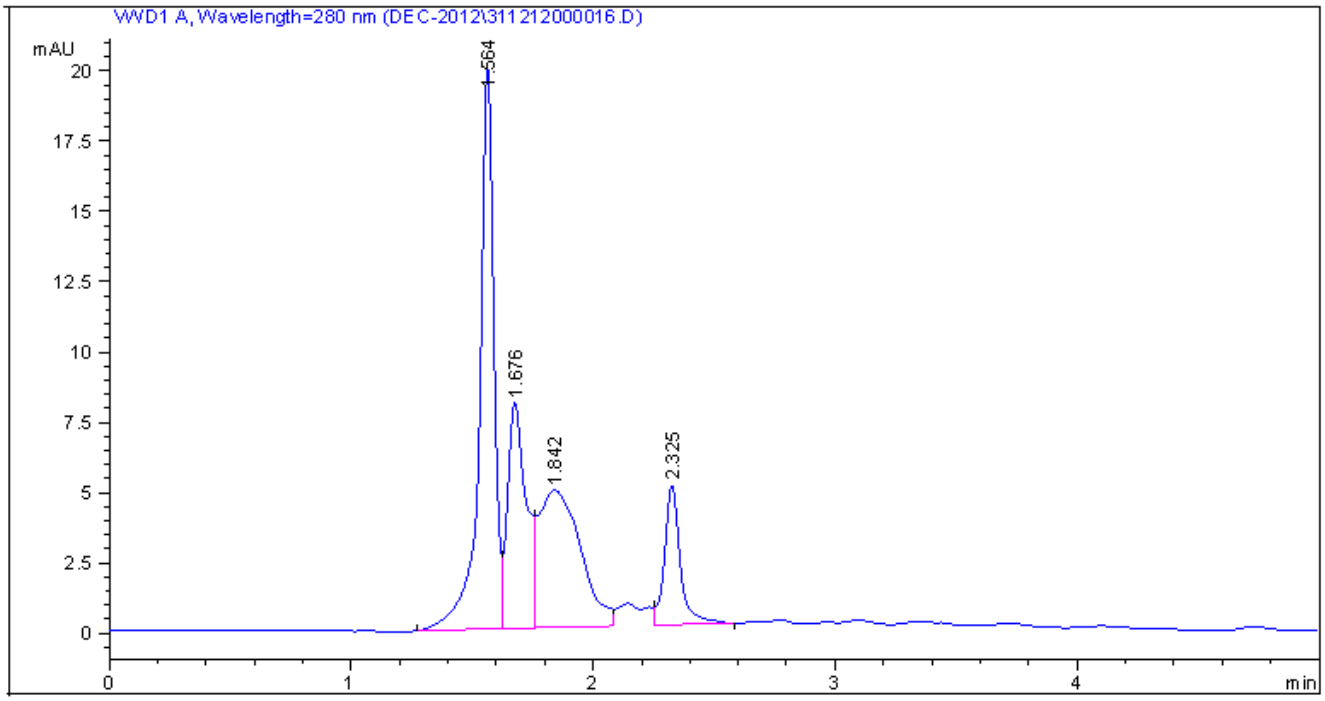


Figure 4
RP- HPLC spectrum of PTLC isolate of EEPL

Qualitative analysis of PTLC isolate was carried out by using RP-HPLC. In addition, chromatographic profile of PTLC isolate was compared with R_t of reference standard. From the chromatographic profile (Figure 3 and Figure 4) it was clearly observed that gallic acid

was present in the PTLC of EEPL. The chromatograms at 280 nm for 1 mg/ml of the reference standard and PTLC isolate showed peaks at R_t of 1.672 and 1.676 respectively. On comparison with standards (Figure 2), a few closely related compounds were found in the PTLC of EEPL at 280 nm. This may be due to the interference of some other phenolic acids in the PTLC of EEPS. Unlike synthetic drugs, herbal medicine is a complicated system of mixtures. Thus, the methods of choice for identification of 'botanical drug' are quite difficult[18]. For such purposes, chromatographic techniques[19] such as HPLC, gas chromatography (GC), and Liquid Chromatography Mass Spectrometry (LC-MS) were widely reported in numerous publications. Although HPLC is the main technique for quality control of identification, isolation and purification. HPLC is found to be very suitable technique for the identification of gallic acid present are in various plants and plant products; Many HPLC finger print studies suggested quality control and standardization because of the ability of good separation and resolution of complex mixtures as well as peak purity control. RP-HPLC was chosen because it is believed to be a most effective analytical technique than normal phase for separation of closely related polar compounds from the plant material. Gallic acids have polar nature and strong chromophores, resulting in RP-HPLC with UV detection being the most common method of analysis. Acetonitrile was used as a mobile phase because of its lower potency of UV absorption and it's high polar nature. However, this is the first chromatographic analysis of gallic acid in the *Polyalthia Longifolia* leaves that need to be investigated further.

CONCLUSION

On the basis of the results of the present study, it was concluded that the gallic acid was successfully isolated from *Polyalthia Longifolia* leaves by PTLC. RP-HPLC analysis also strongly suggested that presence of gallic acid. More research on the purification and structure elucidation of gallic acid in the *Polyalthia longifolia* leaves may be focused and carried out in future.

ACKNOWLEDGEMENT

I would like to express my sincere thanks to Management of Cymbio Pharma Pvt Ltd., Bangalore, Karnataka, India, for their support in carrying the research work. I would like to express my special thanks to Dr.P.Ramalingam, Research Co-ordinator, Raghavendra Institute of Pharmaceutical Education and Research, Anantapur, Andra pradesh.

REFERENCES

1. Rebecca J. R, Phenolic acids in foods: An overview of analytical methodology. J. Agric. Food Chem, 51 (10): 2866-2887, (2003).
2. Siranoush Shahrzad and Irmgard Bitsch, Determination of some pharmacologically active phenolic acids in juices by high-performance liquid chromatography. Journal of Chromatography A, 741: 223-231, (1996).
3. Gupta Mradu, Sasmal Saumyakanti, Majumdar Sohini and Mukherjee Arup, HPLC Profiles of Standard Phenolic Compounds Present in Medicinal Plants. International Journal of Pharmacognosy and Phytochemical Research, 4 (3): 162-167, (2012).
4. Weerasak Samee and Suwanna Vorarat, Simultaneous determination of gallic acid, catechin, rutin, ellagic acid and quercetin in flower extracts of *Michelia Alba*, *Caesalpinia pulcherrima* and *Nelumbo nucifera* by HPLC. Thai Pharmaceutical and Health Science Journal, 2 (2), (2007).
5. Sampath M, Optimization of the extraction process of phenolic antioxidant from

Polyalthia longifolia (Sonn.) Thawaites. Journal of Applied Pharmaceutical Science, 3 (02):148-152, (2013).

6. Ogunbinu A.O, Ogunwande I.A, and Essien E, Sesquiterpenes- Rich Essential Oils of *Polyalthia longifolia* Thw. (Annonaceae) from Nigeria. Journal of Essential Oil Research, 19:419-2, (2007).
7. Ghosh G, Subudhi B.B, Badajena L.D, Mishra S.K, A new clerodane-type γ-hydroxybutenolide diterpene from the bark of *Polyalthia longifolia* var. *angustifolia.* Indian Journal of Chemistry, 50B: 1510-1512, (2011).
8. Sampath, M and Vasanthi, M, Isolation, structural elucidation of flavonoids from *Polyalthia longifolia* (Sonn.) Thawaites and Evaluvation of antibacterial, antioxidant and anticancer potential. International Journal of Pharmacy and Pharmaceutical sciences 2013; 5 (1) 336-341, (2013).
9. Sathish Kumar T, Shanmugam S, Sampath M and Sivachandran S.V, Investigation of antioxidant properties of *Polyalthia Longifolia* leaves. Advanced Biotech, 9: 43-46, (2010).
10. Leonard Vuyani Mabinya, Tungamirai Mafunga and John Morgan Brand, Determination of phenolic acid and related compounds by thin layer chromatography. African Journal of Biotechnology; 5 (13):1271-1273, (2006).
11. Nagore Dheeraj H, Kuber Vinod V, Patil Pankaj S, Sagulale Amol D and Deshmukh Tushar A, A validated RP-HPLC method for quantification of gallic acid as marker in different extracts of *Symplocos racemosa* (Roxb). International Journal of Chromatographic Science, 2 (4): 19-23, (2012).
12. Saikat Sen, Raja Chakraborty, Sridhar C, Reddy Y. S. R and Biplab De, Free radicals, antioxidants, diseases and phytomedicines:Current status and future prospect. International Journal of Pharmaceutical Sciences Review and Research, 3 (1): 94-100, (2010).
13. Murali Krishna. T, Meena. G, Kavya. T, Someshwar. C, Soumya. J Aswaq Ahmed, Rajender. Vadluri and Rajesh Goud. Gajula, *In vitro* determination of anti-oxidant and anti-bacterial activities of *Vitex Negundo* Linn. Int J Pharm Bio Sci, 4 (1): 121 – 127, 2013.
14. Jozwiak G.W and Waksmundzka Hajnos M, Preparative layer chromatography of an extract of alkaloids from *fumaria officinalis*. Acta Chromatographica 2007; 18: 207-218.
15. Karthick K, Kirthiram KS, Sadasivam S, Thayumanavan B and Palvannan T, Identification of α-amylase inhibitor from *Syzygium cumini* (Linn) seed. Indian J Exp Biol, 46: 677-680, (2008).
16. Mahesh Chand Meena and Vidya Patni, Isolation and identification of flavonoid quercetin from *Citrullus colocynthis* (Linn.) Schrad. Asian J. Exp. Sci, 22: 137-142, 2010.
17. Thiyagarajan Sathishkumar, Ramakrishnan Baskar, Mohan Aravind, SuryanarayananTilak, Sri Deepthi and Vellalore Maruthachalam Bharathikumar, Simultaneous extraction optimization and analysis of flavonoids from the flowers of *Tabernaemontana heyneana* by high performance liquid chromatography coupled to diode array detector and electron spray ionization/mass spectrometry. ISRN Biotechnology, 2013: 10, (2013).
18. Angshuman biswas, Ananya Chatterjee and Sandip Kumar Bahdhopadhyay, Quantitative estimation of gallic acid in amla extract by gradient RP-HPLC method. International Journal of Research article Pharmaceutical Innovations. 2 (1):72-76, (2012).
19. Anubhuti Sharma and Priti Gupta, Evaluation of antioxidant activity and validated method for analysis of polyphenols from non-edible parts of Indian tropical fruits by using microwave assisted extraction and LC-MS/MS. Int J Pharm Bio Sci, 4(1): 227 – 241, 2013.