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# Optimization of the Extraction Process of Phenolic Antioxidant from *Polyalthia Longifolia (Sonn.)* Thawaites

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**ABSTRACT**

The research was carried out to optimize the extraction conditions of phenolic antioxidant from the leaves of *Polyalthia longifolia*. It was optimized by using $L_{16}$ orthogonal design of experiment. The effect of single factors such as shaking speed, extraction time, modifier concentration and material ratio on the extraction of the phenolic antioxidant was investigated. The maximum phenolic antioxidant content was obtained under optimum conditions of shaking speed at 200 rpm, shaking time 10 min with material ratio of 1:10 and 80% of ethanol.

## INTRODUCTION

Free radicals may induce oxidative damage to various biomolecules like proteins, DNA, lipids etc., Proteins and enzymes, which are very essential for the human growth, are reduced to a great extent by degradation and other body metabolism also gets affected. Antioxidants are the substances that are used to reduce the oxidative damages and scavenging (Bharathikumar *et al*., 2008) the free radicals by providing the protons. Therefore, ample antioxidants are required to scavenge the excess free radicals. These can be supplemented in dietary intake of food, containing abundant antioxidants (Saikat Sen *et al*., 2010). There is necessity to supplement antioxidant to exterminate free radicals. It can be supplied by either synthetic or natural. Synthetic antioxidants (i.e. Butylated hydroxy anisole, butylated hydroxy toluene, propyl gallate, tertiary butyl hydroquinone etc.,) have shown toxic (Rathore *et al*., 2011) and mutagenic effects, which have stimulated an interest to many investigators to search natural antioxidants from natural sources. Recent researches have shown that the antioxidants of plant origin with free-radical scavenging properties have great importance as therapeutic agents in several diseases caused due to the formation of free radicals (Mark Percival, 1998). Plant extracts and phenolic compounds are found as an effective free radical scavengers or inhibitors. Therefore, abundant phenolic compound producing biological material, which is required to fight against with the free radicals. Phenolic acompounds are secondary metabolites of plants. Generally, they were synthesized (Farah and Donangelo, 2006) in high stress conditions such as ultraviolet radiation, pathogens attack etc., and several thousands of phenolic compounds have been described in plant origin (Rathore *et al*., 2011). Most of these compounds have received considerable attention as potentially protective factors against human chronic degenerative diseases like, neurodegenerative diseases, diabetes mellitus, cancer and cardiovascular disease. *Polyalthia longifolia* is a lofty evergreen tree, native to India. This genus of the plants generally found in the tropical and subtropical parts of India up to an altitude of 1500 m. It is introduced in gardens in many places around the India. *Polyalthia longifolia* is identified and is chosen as a source of material because it is a well-known stress tolerant plant. Moreover it contains (Padmaa *et al*., 2009) important phenolic compounds like flavonoids, alkaloids, terpenoids and saponins.

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Several biological activities like anti ulcer, anti diabetics, anti microbial, anti cancer, anti pyretic and anti inflammation has been reported from *Polyalthia longifolia* species (Katkar *et al*., 2010). Various antioxidant activities (Moni Rani *et al*., 2008) of different parts of plant such as seed, stem, bark and leaves from *Polyalthia Longifolia* were already reported. Chromatographic analysis (Sampath and Vasanthi, 2013) of the ethanolic extract of *Polyalthia longifolia* leaves has revealed the presence of phenolic antioxidant such as Rutin, Chrysin and Daidzin related isomers.

However, in Indian scenario many researchers have been reported that the extraction of phenolic antioxidant from various plant sources. But unfortunately, there are no more scientific documentation on the optimization of extraction conditions of phenolic antioxidant from plant sources. In many cases, Prediction of extraction conditions for plant metabolites are not yet straightforward (Jahanshashi *et al*., 2008). Therefore, good experimental design is necessary for extraction process. From past three decades (Sterbova *et al*., 2004) Plackett-Burnman design and Response surface methodologies are offnely used to optimize extraction conditions for plant metabolites. Recent years, orthogonal design of experiments (Sathish Kumar *et al*., 2009) are simple, rapid, economical and designed for optimize the extraction conditions for plant metabolites. The present study is an attempt that has been made to optimize the extraction conditions of total phenolic antioxidant from ethanolic extract of *Polyalthia lonifolia* leaves.

## MATERIALS AND METHODS

### Sample Collection

The leaves of the plants were collected from the medicinal garden of Cymbio Pharma Private Limited, Bangalore, Karnataka, India, identified by an expert taxonomist and confirmed by Botanical Survey of India (BSI), Southern Circle, Coimbatore, India. The voucher specimen of the sample (SAM-05) was deposited at the Department of Research and Development, Cymbio Pharma Private Limited., Bangalore, Karnataka, India.

### Extracting parameters

At first the main factors of the extraction temperature, the concentration of the extracting agent, extraction time and the materials ratio (weight of leaves: volume of the extracting agent), which affect the extraction of phenolic antioxidant were studied individually, and then the optimum extracting conditions of phenolic antioxidant from raspberry fruits were determined by adopting $L_{16}(4^4)$ orthogonal experiments. A single factor analysis of variance (One way ANOVA) was espoused to investigate the effect of each factor in the extraction of phenolic antioxidant.

### Estimation of total phenolic antioxidant by phosphomolybdenum method

0.2ml (concentration varying from 5 to 50µg) of the rutin was added (Pilar *et al*., 1999) in all the test tubes. To all the tubes, including the blank, distilled water was added to make up to 2.0ml. To all the tubes, 2ml of phosphomolybdenum reagent was added and incubated for 90 minutes at 90°C. After the mixture was cooled to room temperature, the absorbance of each solution was measured at 695 nm against a blank. The antioxidant capacity (Fig.1) was expressed as rutin equivalent.

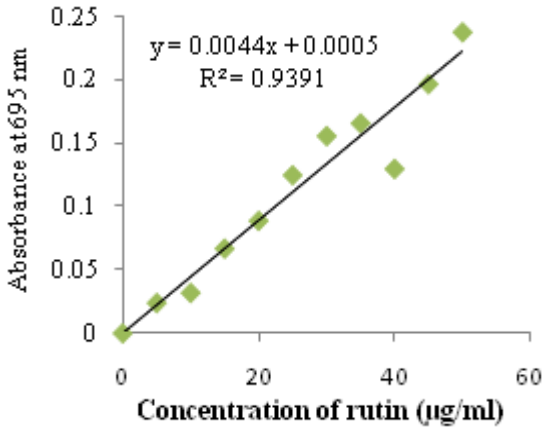


**Fig.1:** Standard curve for estimation of antioxidant content.

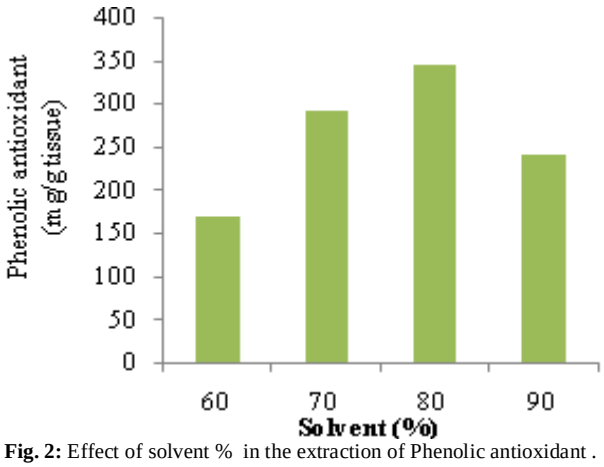


**Fig. 2:** Effect of solvent % in the extraction of Phenolic antioxidant .

## RESULTS AND DISCUSSTION

### Effect of solvent concentration in the extraction of phenolic antioxidant

When the solvent concentration was increased, the fluid polarity will also get enhanced. This helps in the solublize the polar nature compounds from the plant material. Generally, most of the plant derived phenolic antioxidant compounds are polar in nature. Here ethanol was used as a modifier. It interacts with the phenolic antioxidant probably through non-covalent interactions and promotes a rapid diffusion into the solution. The contents of phenolic antioxidant gradually increased with a rise in the ethanol concentration (Fig.2) in a range of 60% to 80%.When concentration reaches at 90% a sudden decrease was observed in the extraction of phenolic antioxidant content. This may due to the interaction of non-phenolic compounds in the plant material. Recently (Sathishkumar *et al*., 2009) reported that 75% of ethanol concentration was optimum for the extraction of maximum flavonoids from the *polyalthia longifolia (sonn) Thawaites*. The

similar study has been carried out by (Waksmundzka-Hajnas *et al.*, 2004) has proved the maximum yield of Furanocoumarins from *Pastinaca sativa* fruits attained at 80% of methanol concentration. According to (He Guo-qing *et al*., 2004) higher the fluid polarity increased will disturb the structural stability of the plant cell wall also, it contributes to the extraction of non targeted biomolecules like protein, sugars, fattyacids, aminoacids etc.,

**Effect of solid: liquid (W/V) in the extraction of phenolic antioxidant**

A correct ratio of solvent and plant matter is fundamental for obtaining an optimal extraction process. The phenolic antioxidant was extracted at different material ratio from 1:5 to 1:20 (W/V). When the solvent volume was increased, it can increase the absorption rate, swelling rate and diffusion rate of the plant cell wall. At the same time excessive solvent volume, promotes the extraction of undesired compound from the plant material. This may affect the quality of the desired compounds and decrease the yield also. The maximum (Fig.3) phenolic antioxidant was obtained at 1:10. The phenolic antioxidant content gradually decreases with a rise in the material ratio in a range of 1:15 to 1:20. This decrease due to the fact that when the ratio of solvent to raw material reached a certain level, the extract may be well saturated (Yaqin *et al.*, 2005) with the solutes in the solution that may lead the extract to reach a steady and may not increase significantly furthermore. Thus, this study proved that higher solvent volume will give lower yield.

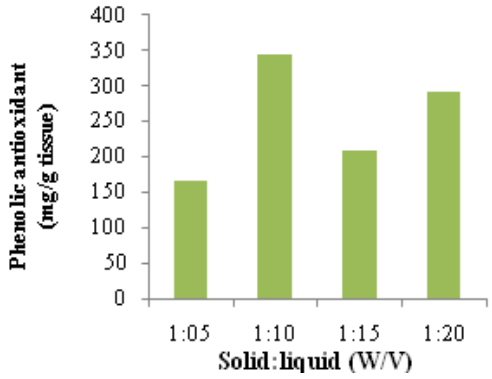


**Fig. 3:** Effect of solid:liquid (W/V) in the extraction of phenolic antioxidant.

**Effect of shaking speed (rpm) in the extraction of phenolic antioxidant**

The phenolic antioxidant content gradually (Fig.4) increased with a rise in the shaking speed. Majority of the extraction procedure i.e solvent, centrifugal assisted, supercritical fluid extraction etc., diffusion is the most possible mechanism. The traditional techniques of solvent extraction of plant materials are mostly based on the correct choice of solvents and the use of heat or/and agitation to increase the solubility of the desired compounds and improve the mass transfer (Vivekananda Mandal *et al.*, 2007). According (Fossing *et al*., 1995) to the Ficks law, when molecules move from high concentration to low concentration through semi permeable membrane that needs appropriate driving force (Kain *et al*., 2009). Here shaking effect used as the driving force. The basic mechanisms is that the increasing driving force could increase the mass transfer rate and facilitate the concentration gradient between inside and outside plant cells, which consequently prompted diffusion rate of solute particles and made more phenolic antioxidants enter to the solution. Thus, present study strongly suggested that the shaking effect is the promising factor for the extraction of maximum yield of phenolic compounds from the plant material.

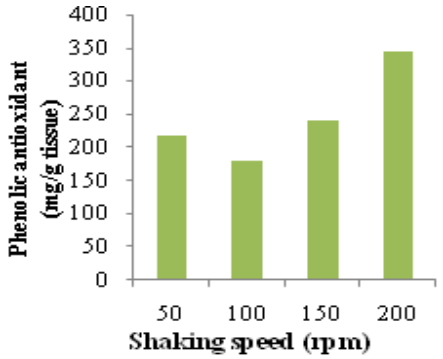


**Fig. 4:** Effect of shaking speed in the extraction of Phenolic antioxidant content.

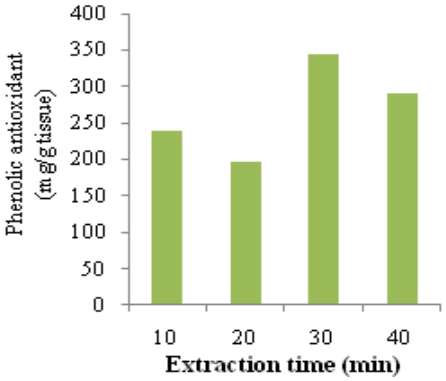


**Fig. 5:** Effect of extraction time in the extraction of Phenolic antioxidant.

**Effect of extraction time in the extraction of phenolic antioxidant**

The result of (Fig.5) showed that the content of phenolic antioxidant extracted at 30 min reached maxima and prolonged extraction may not give increased yield. Furthermore, a decrease in the phenolic antioxidant was noticed at above 30 min extraction. Previous report has been proved (Sterbova *et al*., 2004) that 30 min was required for the extraction of phenolic compounds from *Hypericum perforatum* and *Thymus vulgaris*. The plant cell wall consists of strong, tight and high cellulose moiety (Valent and Albersheim, 1974). This makes the structural stability of the plant cell wall. It requires appropriate organic solvent and higher extraction time to destabilize/solublize the strong cellulose structure. The fact is that one single plant can contain up to several thousand secondary metabolites, makes the need for the development of high performance and rapid extraction methods an

absolute necessity (Nyiredy, 2004). Generally, when the extraction time is increased, it will increase the extraction yield also. At the same time prolonged exposure of solvent assistance, affects the analyte nature, stability and contribute the extraction of impurities. It occurs at an increased extraction time at which some components of the material were separating or reacting with one another to modify the macrostructure or microstructure (Luque De Castro and Garcia-Ayuso, 1998).

**Table. 1: (**4)$^4$ orthogonal design parameters.

| Levels | A Solvent (%) | B Solid: liquid ratio (W/V) | C Shaking speed (rpm) | D Extraction time (min) |
|---|---|---|---|---|
| 1 | 60 | 1:5 | 50 | 10 |
| 2 | 70 | 1:10 | 100 | 20 |
| 3 | 80 | 1:15 | 150 | 30 |
| 4 | 90 | 1:20 | 200 | 40 |

**Table. 2:** One way ANOVA analysis.

| Levels | Sum of square | Degrees of freedom | Mean square | F-value |
|---|---|---|---|---|
| A | 16314.93 | 3 | 5438.31 | 1.035 |
| B | 32974.01 | 3 | 10991.34 | 2.843 |
| C | 13552.268 | 3 | 4517.42 | 1.213 |
| D | 13086.80 | 3 | 4362.26 | 1.266 |

**Table. 3:** Experimental results and range analysis.

| EXP. | A | B | C | D | Total antioxidant content (mg/g tissue) |
|---|---|---|---|---|---|
| 1 | 1 | 1 | 2 | 3 | 66.26 |
| 2 | 1 | 2 | 1 | 4 | 169.74 |
| 3 | 1 | 3 | 4 | 1 | 147.57 |
| 4 | 1 | 4 | 3 | 2 | 133.04 |
| 5 | 2 | 1 | 1 | 1 | 120.55 |
| 6 | 2 | 2 | 2 | 2 | 156.15 |
| 7 | 2 | 3 | 3 | 3 | 209.76 |
| 8 | 2 | 4 | 4 | 4 | 290.68 |
| 9 | 3 | 1 | 3 | 4 | 166.39 |
| **10** | **3** | **2** | **4** | **3** | **344.47** |
| 11 | 3 | 3 | 1 | 2 | 196.73 |
| 12 | 3 | 4 | 2 | 1 | 155.31 |
| 13 | 4 | 1 | 4 | 2 | 66.625 |
| 14 | 4 | 2 | 3 | 1 | 239.43 |
| 15 | 4 | 3 | 2 | 4 | 179.48 |
| 16 | 4 | 4 | 1 | 3 | 216.59 |
| K1 | 516.61 | 419.82 | 703.61 | 662.86 | 66.26 |
| K2 | 777.14 | 909.78 | 557.2 | 552.54 | 169.74 |
| K3 | 862.9 | 733.54 | 748.62 | 837.08 | 147.57 |
| K4 | 702.12 | 795.62 | 849.34 | 806.29 | 133.04 |
| $k_1$ | 129.15 | 104.95 | 175.90 | 165.71 | 120.55 |
| $k_2$ | 194.28 | 227.44 | 139.3 | 138.14 | 156.15 |
| $k_3$ | 215.72 | 183.38 | 187.15 | 209.27 | 209.76 |
| $k_4$ | 175.53 | 198.90 | 175.9 | 201.57 | 290.68 |
| R | 86.57 | 122.49 | 47.85 | 71.19 | |

**K**- Values obtained from individual factors (solid: ratio, solvent % etc.,); **R**-Rank,

**Optimum conditions for the extraction of phenolic antioxidant**

The parameters and the orthogonal design of experiment for the extraction of phenolic antioxidants were given in the Table 1. The results were made in the form of range analysis and one way ANOVA by Sigmastat 3.5 software. The results were depicted in Table 2 and Table 3. The order of the effect of factors on phenolic antioxidant extraction was B>D>C>A. The material ratio was observed to possess a greatest effect on the extraction procedure and the extraction time was found to be a secondary parameter even though it was not proved to be a significantly different at 5% level.

## CONCLUSION

In summary, the extraction of phenolic antioxidant was successfully optimized by $L_{16}$ orthogonal design of experiment. The optimal condition for the extraction of phenolic antioxidant content was found to be at 200 rpm, for 30 min extraction duration, 80% ethanol and 1:10 material ratio. The investigation proved that the Solid: liquid ratio was found to be a major factor that affects the extraction procedure of phenolic antioxidant content. More research on the purification and structure elucidation of phenolic antioxidant in the *Polyalthia longifolia* leaves may be focused and carried out in future.

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