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# Quantitative Determination of Total Content of Phenol, Flavonoid And Tannin In Leaf Extract of *Barlaria Buxifolia* Linn

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## ABSTRACT

*Barlaria buxifolia* Linn is one of the medicinal plants well documented traditionally in Ayurveda system of medicine and is highly valued in modern medicine owing to the presence of alkaloids, flavonoids, tannins, phenolic compounds, steroids. The plant is reported to contain phenol, flavonoid and tannin; phenolic and flavonoids compounds are reported to possess antioxidant and hence the plant may be used as organ protective. Keeping this in view, the plant was analysed for total phenol, flavonoid and tannin content. Catechol, quercetin and tannic acid reagents were used as standards for calibration of total polyphenols, flavonoids and tannins respectively. The quantification of total polyphenol, falvonoid and tannin content showed 14.65mg/gm catechol, 26.80mg/gm quercetin and 11.32mg/gm tannic acid equivalent respectively, the study indicates that the leaves of *Barlaria buxifolia* Linn exhibits the highest flavonoid, phenolic and tannin content. It can be used potentially as a readily accessible source of natural antioxidant.

**Key words:** Catechol, Phenol, Flavonoid, Quercetin and Tannin, *Barlaria buxifolia* Linn

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## INTRODUCTION

Antioxidants are the substances which chemically react with free radicals and render them harmless and at the same time break the viscous circle, which involve in the decomposition of fatty acids and proteins, the creation of new free radicals and leads to eventual cell death[1]. Antioxidants reduce the energy of the free radical; suppress radical formation, as radical scavengers (break chain propagation), help in repair damage and reconstituted membranes. These are nutraceuticals, whose deficiency associated with numerous dreadful disease conditions viz, cardiovascular disease, diabetics, rheumatoid arthritis and Alzheimer disease nephro and neurological disorders[2]. Majority of the diseases are mainly linked to oxidative stress due to free radicals. Continuous exposure to stressful conditions generates free radicals, which may over power the inbuilt protective mechanism and tissue damage. The most important free radicals in many disease states are superoxide anion ($O_2^{\bullet}$), $H_2O_2$ radical, NO radical, hydroxyl ion radical ($OH^{\bullet}$), NOO, etc. These are highly reactive species, in the nucleus and in the membranes of cells damage the biologically relevant molecules such as DNA, proteins, carbohydrates and lipids[3].

Although the antioxidant defense systems includes both endogenously and exogenously derived compounds, dietary plants based antioxidant have recently received a great attention. Hence many studies have been performed to identify antioxidant compounds with pharmacological activity and a limited toxicity from medicinal plants[4]. Antioxidants may play an important role in the chronic disease prevention by arresting oxidative damage caused by reactive oxygen species (ROS) to vital biomolecules such as DNA, lipids, and proteins[5].

Medicinal plants are believed to be an important source of new chemical substances with potential therapeutic effects. *Barlaria buxifolia* Linn (Acanthaceae) is widely distributed throughout the greater part of India[6,7,8,9]. This plant has useful medicinal properties[10] and extracts of the roots and leaves of this plant are known to be used locally for curing cough and inflammation[11]. Different parts of *Barlaria* species have been used traditionally for the treatment of variety of disease including anemia, toothache and as a hypoglycemic agent. Moreover, the plant species is used to treat stiffness of the limbs, enlargement of the scotum and sciatica. Ethanol extract of *Barlaria buxifolia* Linn stem showed antimicrobial and antituberculosis activity[12]. The plant is reported to contain phenolic compounds; phenolic compounds are known to be antioxidants and are reported to have organ protective role[2]. However there is no literature found on quantification of phenol, flavonoids and tannins. Hence the present study has been under taken with aim to quantitative determination of total content of phenol, flavonoid and

tannin of ethanol extract of *Barlaria buxifolia* Linn leaves.

## MATERIALS AND METHODS

### Collection of plant material and preparation of extracts

Fresh leaves of the plant *Barlaria buxifolia* Linn were collected from the local areas of Nelamangala taluk, Bangalore rural district, Karnataka, India during the month of May-June and was taxonomically identified by Dr. Krishnegowda, Director, Dayanandasagar college of biological sciences, Bangalore. The dried powder of the leaves was extracted with ethanol using soxhlet apparatus. The extract was concentrated under reduced pressure using rota flash evaporator and stored in airtight containers in refrigerator below 10ºC.

This extract was screened for the presence of various secondary metabolites mainly tannins, flavonoids, polyphenols, steroids and glycosides using standard qualitative tests. This extract was subjected for quantification of their total phenolic, flavonoid and tannin content.

### I. Quantification Studies:

### Total Phenolic Content (TPC)

The total phenolic content was determined by adopting the method as described in Malik E.P and Singh M.B et al[13]. Aliquots of the extract was taken in a 10 ml glass tube and made up to a volume of 3 ml with distilled water. Then 0.5 ml Folin ciocalteau reagent (1:1 with water) and 2 ml $Na_2CO_3$ (20%) were added sequentially in each tube. A blue color was developed in each tube and the intensity of the color is directly proportional to the phenolic content. The blue coloration in the tube is due to the formation of molybdenum blue as a result of complex redox reaction between phenols and phosphomolibdic acid in Folin ciocalteau reagent in alkaline medium. The test solutions were warmed for 1minute, cooled and absorbance was measured at 650 nm. The calibration curve was prepared using catechol. The phenolic content of the plant was expressed as a mg. equivalent of phenol per gm. of extract.

### Total Flavonoid content (FC)

The total flavonoid content was determined by adopting the method as described in Heljimaet al[13]. Aliquots of each extract was pipetted out in series of test tubes and volume was made up to 0.5ml with distilled water; sodium nitrite (5%: 0.3ml) was added to each tube & incubated for 5 min. at room temperature; aluminium chloride solution (10%; 0.06ml) was added and incubated for 5 min, at room temperature; Sodium hydroxide (1M; 0.25ml) was added and total volume was made to 3ml with distilled water. Absorbance was measured at 510 nm against a reagent blank using Shimadzu model 1700 double beam spectrophotometer and concentration of

flavonoids in the test sample was determined and expressed as mg equivalent of quercetin per gram of sample.

**Total Tannins content (TC)**

The tannin content was determined by using $FeCl_3$ and gelatin tests[14]. 0.1g of the leaves extract was transferred to a 100ml flask; 50ml of water was added and boiled for 30min. After filtration with cotton filter, filtrate was transferred to a 500ml flask and the volume was made up to the mark with distilled water. 0.5 ml aliquots were transferred to vials, 1ml of 1% $K_3Fe(CN)_6$ and 1 ml of 1% $FeCl_3$ were added and the volume was made up to 10ml with distilled water. After 5 min absorbance was measured at 510 nm against a reagent blank using Shimadzu model 1700 double beam spectrophotometer and concentration of tannins in the test sample was determined and expressed as mg equivalent of tannic acid per gram of sample.

## RESULTS AND DISCUSSION

Plants are conceived as sources of antioxidants due to presence of polyphenols, flavonoids and tannins which possess wide biological properties. Recent studies showed that these constituents contribute significantly to the total antioxidant activity of many plants. The preliminary phytochemical screening of the extract showed that they contain phenols, flavonoids, steroids, tannins and glycosides. However it was observed that the total content of Phenols, flavonoids and tannins was found to be very much higher than the non polar constituents like steroids. TPC, FC and TC of ethanolic extract was found to be 14.65mg/gm, 26.80mg/gm and 11.32mg/gm interms of catechol, quercetin and tannic acid equivalent respectively and the results are compiled in Table I .The standard curves for catechol, quercetin and tannic acid were depicted in the figures I, II and III respectively. These reports are indicating that total phenolic, flavonoid and tannin content is directly proportional to antioxidant activity of the leaves of the plant.

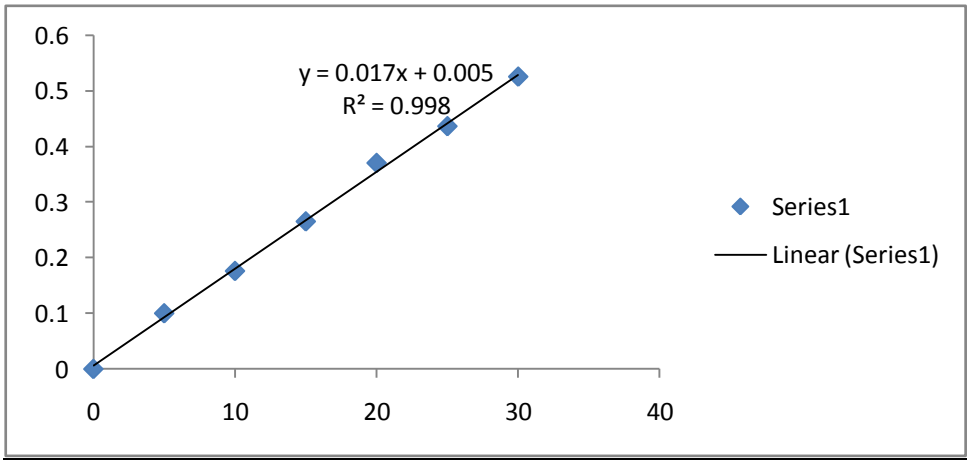


**Figure I: Catechol Calibration Curve at 650nm**

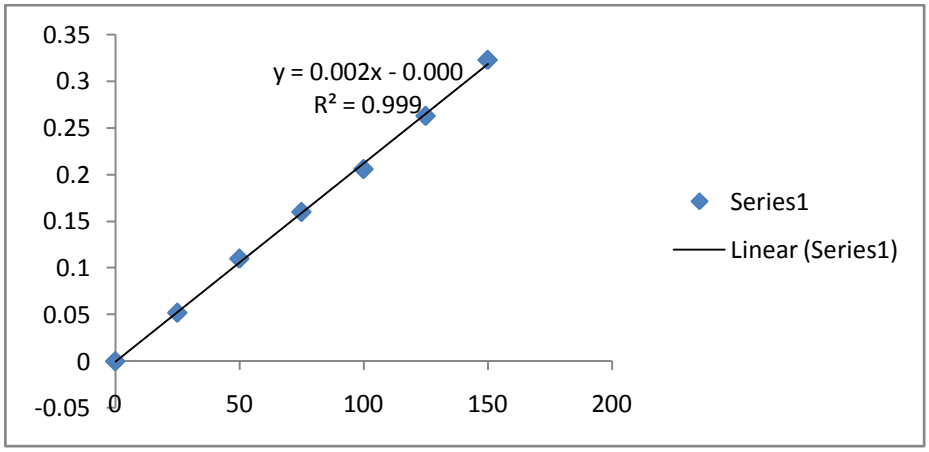


**Figure II: Quercetin Calibration Curve at 510nm**

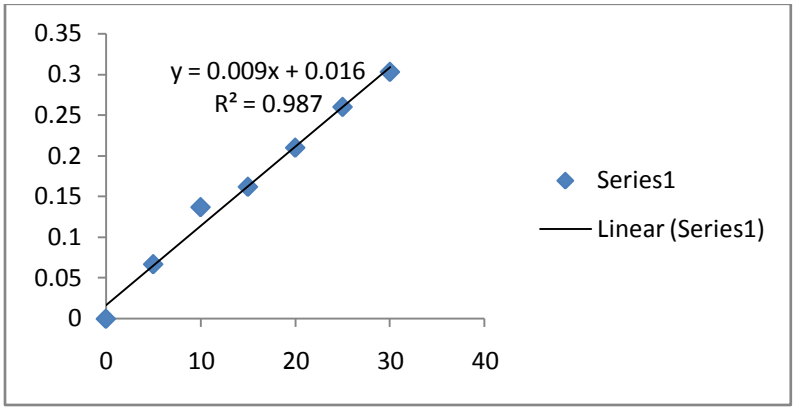


**Figure III: Tannic acid Calibration Curve at 700nm**

**Table I:Total phenolic, flavonoid and tannin content of ethanolic extract**

| Sl. No | Name of the secondar metabolite | Absorbance At | mg equivalent |
|---|---|---|---|
| 1 | Phenols | 650nm | 14.65mg/gm Catechol equivalent |
| 2 | Flavonoids | 510nm | 26.80mg/gm Quercetin equivalent |
| 3 | Tannins | 700nm | 11.32mg/gm Tannic acid equivalent |

## CONCLUSION

In the present study the total phenol, flavonoid and tannin content were determined and this in terms helps in gauging the antioxidant potential of the leaves of the plant. In addition to this the present findings are not only helpful for establishing the phytochemical standardization but also in authentication of this drug. However further studies are needed to assess the organ protective potential of the plant.

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