

Quantitative determination of phytochemical constituents from *Anisomeles malabarica*

Abstract

Background: Plant derived secondary metabolites make up a significant segment of natural product based pharmaceuticals especially from the herbal medicinal plants. Hence, it is of interest to investigate the quantification of Phytochemicals such as total content of alkaloids, flavonoids, tannins and phenolic compounds are of our interest.

Methods: Quantitative analysis of phytoconstituents of chloroform extract of *A. malabarica* was used to find out the quantification of Phytochemicals.

Results: The results of the present study envisage that the presence of alkaloids, flavonoids, tannins and poly phenol compounds.

Conclusion: The chloroform extract of *Anisomeles malabarica* contains pharmacologically active phytochemical constituents.

Keywords: phytochemical quantification, *anisomeles malabarica*, tannins, phenols, flavonoids

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Introduction

Traditional system of Indian medicine is an ancient system with thousands of medicinal plants for the healthcare applications. At present, many developed nations are moving to make use of the herbal medicinal systems due to its phyto constituents are efficacy in many therapeutic applications. Since, they show protective mechanism against diseases such as human malignancies, heart diseases, diabetes melitus, neuronal diseases. Plant-based molecules are also concerned in anti viral and antimicrobial activity. Strategy of engineered phytochemicals is developed to promote solubility, cellular permeability, proteolytic stability and self life of plant based compounds further study is needed to ensure maximum yield and viability as well as bio availability of medicinal plants derived compounds in various medical applications.¹

Anisomeles malabarica is available in Western Ghats, southern India. This plant is considered to be an important medicinal plant because of its phytochemical potential.² *Anisomeles malabarica* (L.) R. Br. is a highly aromatic plant belonging to the family Lamiaceae (Labiatae). *Anisomeles malabarica* is a species of herbaceous plant native to tropical and subtropical regions. Mosquitoes act as a vector for most of the life threatening diseases like malaria, yellow fever, dengue fever, filariasis, encephalitis³

Materials and methods

Samples collection

The present study *Anisomeles malabarica*, whole plant parts, were used and the plant collected from the medicinal farms, Chennai, India and the parts of the plant authenticated by botanist.

Preparation of extracts

1000grams of plant material was packed in three separate round bottom flask for sample extraction using solvents namely Aqueous, Chloroform and Methanol. The extraction was conducted by 250ml of the each solvent mixture for a period of 24hours. At the end of the extraction the respective solvents were concentrated under reduced pressure and keep it in water bath (at 50°C). Now the extracted experimental solutions were stored in refrigerator.

Chemicals and reagents

All chemicals were used for this project were purchased from M/s. Sigma Chemicals, USA.

Quantification of phytochemicals

Determination of total phenolic content, Determination of total tannin Content, Determination of total Alkaloid content and Determination of total flavanoid content were analysed.⁴⁻¹⁶

Quantification of total content of alkaloids

1 mg of the plant extract was dissolved in dimethylsulphoxide) and added 1ml of 2N HCl and filtered. This solution was transferred to a separating funnel, 5ml of bromocresol green solution then 5ml of phosphate buffer were added. The mixture was shaken with 1, 2, 3 and 4ml of chloroform by vigorous shaking and collected in a 10ml volumetric flask and diluted to the volume with the chloroform. A set of reference standard solutions of atropine (20, 40, 60, 80 and 100µg/ml) were prepared in the same manner as described already. The absorbance for standard solutions and test solutions were determined on the reagent blank at 470nm with an UV/Visible spectrophotometer. The content of alkaloids was expressed as mg of AE/g of plant extract.

Total content of flavonoids quantification.

Colorimetric assay was used to determine the total content of flavonoid using aluminium chloride For the reaction, the plant extract of 1 ml and distilled water of 4 ml was taken in a 10 ml of flask. 0.30 ml of 5 % sodium nitrite and after 5minutes, 0.3ml of 10 % aluminium chloride was mixed in the flask. 5minutes later, 2 ml of 1M NaOH was treated and diluted using 10 ml distilled water. A set of standard solutions of quercetin (20, 40, 60, 80 and 100µg/ml) were prepared as mentioned earlier. The absorbance was measured for test and standard solutions using reagent blank at 510nm wavelength by UV-Visible spectrophotometer. The total content of flavonoid was denoted as mg of QE/g of extract.

Quantification of tannin total content

Folin-Ciocalteu method was used to quantify the tannin total content. About 0.1ml of plant extract was added in 10 ml of volumetric

flask containing the distilled water of 7.5ml and Folin-Ciocalteu phenol reagent of 0.5ml, 35% Na_2CO_3 solution of 1 ml and diluted to 10ml using distilled water. The reagent mixture was well shaken and kept at 30°C temperature for 30min. A set of gallic acid solutions (20, 40, 60, 80 and 100 $\mu\text{g}/\text{ml}$) were prepared as mentioned earlier. Absorbance of standard and test solutions was analyzed with blank at 725nm wavelength using UV-Visible spectrophotometer. The tannin total content of tannin was expressed as mg of GAE/g of extract

Quantification of total content of phenolic compounds

The phenolic compounds concentration in extract was quantified by Spectrophotometry method. Folin-Ciocalteu method was employed for the quantification of total phenolic content. The reaction mixture contains 1 ml of plant extract and 9ml of distilled water. 1 ml of Folin-Ciocalteu phenol reagent was treated with the mixture and well shaken. After 5minutes, 10 ml of 7 % Na_2CO_3 solution was treated with the mixture. The volume was 25ml. A set of gallic acid standard solutions (20, 40, 40, 60, 80 and 100 $\mu\text{g}/\text{ml}$) were prepared as earlier. Incubated for 90 min at 30°C and absorbance was analyzed for test and standard solutions with reagent blank at 550 nm with using UV-Visible spectrophotometer. The content of total phenolic compound was denoted as mg of GAE/gm of extract.

Results and discussion

The present study was performed to evaluate the total content of phenols, tannins, alkaloids and flavonoids in chloroform extract of *Anisomeles malabarica* (Figure 1–8). Figure 1 & Figure 2 shows that the total alkaloids content in standard and chloroform of extract of *Anisomeles malabarica*. Total alkaloids content in the extract expressed in terms of atropine equivalent (mg of AE/g of extract) ($y=63.45x-29.75$, $R^2=0.681$), ($y=97.63x-99.90$, $R^2=0.842$). Figure 3 and Figure 4 shows that the total flavonoids content in standard and chloroform of extract of *Anisomeles malabarica*. Total flavanoid content in the extract denoted in terms of quercetin equivalent (mg of Q E/g of extract) ($y=0.001x+0.806$, $R^2=0.890$), ($y=0.001x+0.847$, $R^2=0.929$). Figure 5 and Figure 6 shows that the total tannins content in standard and chloroform of extract of *Anisomeles malabarica*. Total Tannins content in the extract expressed in terms of gallic acid equivalent (mg of GAE/g of extract. ($y=0.000x+1.28$, $R^2=0.901$), ($y=0.001x+0.93$, $R^2=0.957$). Figure 7 & Figure 8 shows that the total phenolic compound content in standard and chloroform of extract of *Anisomeles malabarica*. Total phenolic contents in the extract expressed in terms of gallic acid equivalent (mg of GAE/g of extract) ($y=0.000x+1.888$, $R^2=0.960$), ($y=0.000x+1.779$, $R^2=0.904$). The Pharmacological, Toxicological and Biochemical mechanism of action of chloroform extract of *Anisomeles malabarica* was determined and which are responsible for the therapeutic properties.¹⁷

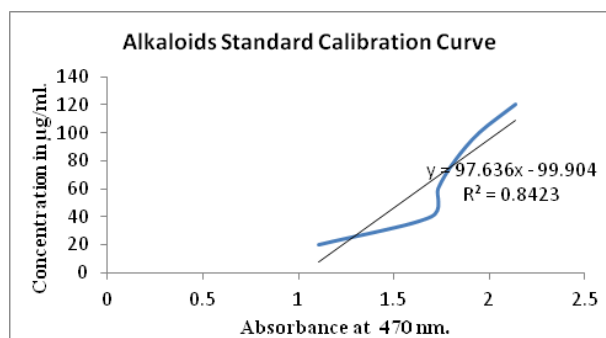


Figure 1 Standard calibration curve of total content of Alkaloids.

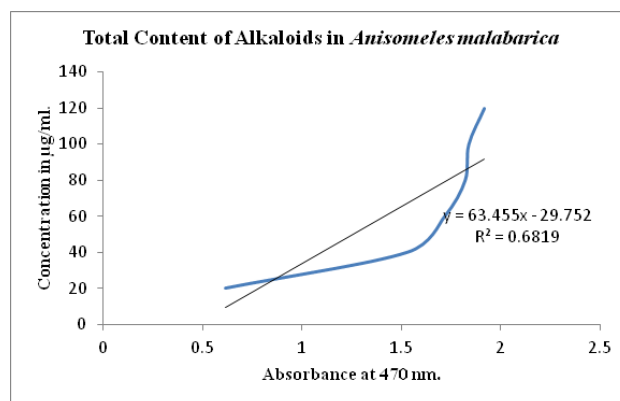


Figure 2 The total content of alkaloids in *A.malabarica*.

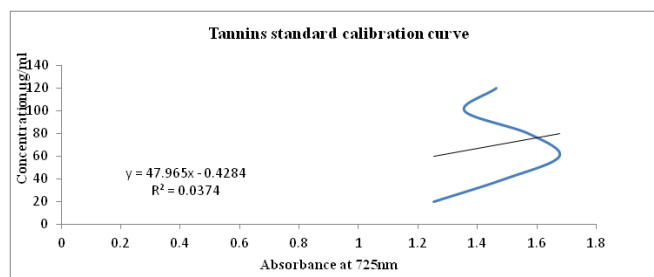


Figure 3 Standard calibration curve of total content of Tannins.

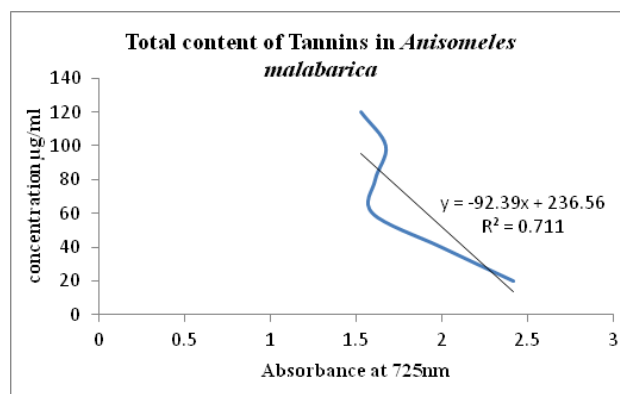


Figure 4 The total content of Tannins in *A.malabarica*.

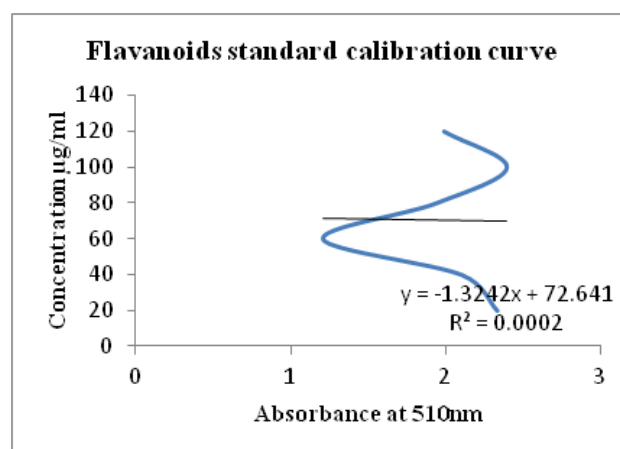


Figure 5 The standard calibration curve of total content of Flavonoids.

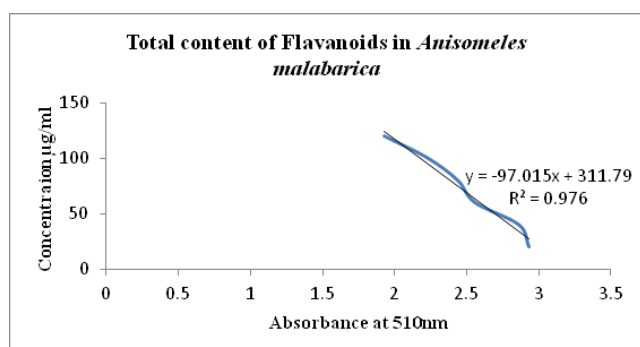


Figure 6 The total content of Flavanoids in *A. malabarica*.

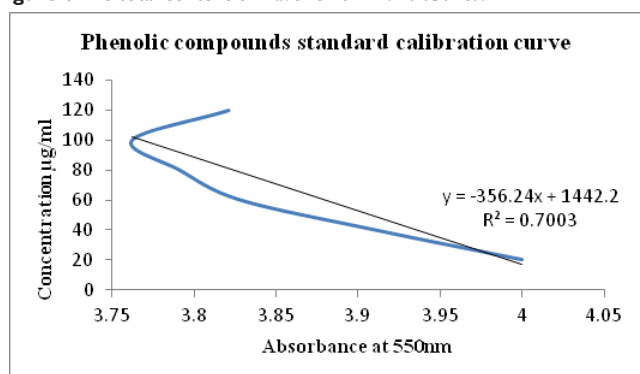


Figure 7 The standard calibration curve of total content of Phenolic compounds.

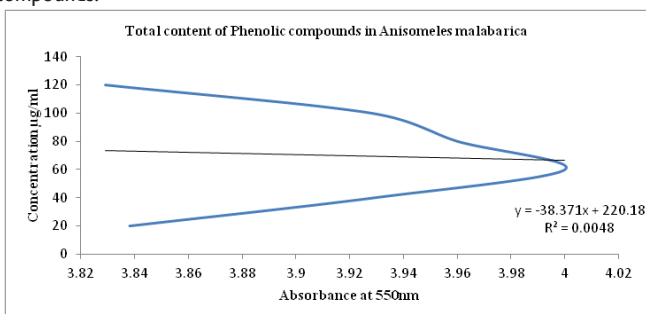


Figure 8 The total content of Phenolic compounds in *A. malabarica*.

Acknowledgments

None.

Conflicts of interest

The author declares there are no conflicts of interest.

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