Research Article

Quantitative Analysis of Phytoconstituents of Various Extracts of Poly Herbal Formulation *Nalla marunthu*

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ABSTRACT

The objective of the study is to determine the quantitative analysis of the phyto components such as total contents of Phenolics, Tannins, Alkaloids and Flavonoids from various extracts of a poly herbal formulation of *Nalla marunthu. Nalla marunthu.* A poly herbal powder material was carried out by continuous hot percolation method in soxhlet apparatus using as chloroform, ethyl acetate and methanol solvents. Gallic acid was used as standard for the determination of total phenol and tannin by Folin -ciocalteu method. Total alkaloid content was determined by chloride colorimetric method using quercetin as a standard. The results showed that ethyl acetate extract has high concentration of total phenol, tannin, alkaloid and flavonoid contents as compared by bromocresol green solution using atropine as a standard. Total flavonoid content was determined by chloroform, ethyl acetate and methanol extracts. Chloroform extract contained the total phenol of 40.20 and tannins of 76.06 as mg of gallic acid equivalents (GAE), alkaloids of 58.20 as mg of atropine equivalents (AE) and flavonoids of 62.32 as mg of quercetin equivalents (QE). In this study, total phenol, tannin, alkaloid and flavonoid contents from chloroform, ethyl acetate and methanol extracts of a herbal formulation of *Nalla marunthu* was screened. Chloroform extract of an herbal formulation *Nalla marunthu* were showed high contents of phenol, alkaloid, flavonoid and tannin as compared to ethyl acetate and methanol extract.

Keywords: *Nalla marunthu* Total alkaloid content, Total content of flavonoid, Poly phenol content, Tannin content.

INTRODUCTION

The secondary plant metabolites were found to be source of various phyto components that could be directly used as intermediates for the production of new drugs. Traditional medicine should be able to play an even greater role in the modern primary healthcare system of the developing countries. The natural medicines are believed to be more acceptable to the human body, when compare to modern synthetic drugs. Thus the most important factor needed is to derive the maximum benefit from the traditional system of medicine for providing adequate healthcare service to rural people. Nature has long been an important source of medicinal agents. An impressive number of modern drugs have been isolated or derived from natural source, based on their use in traditional medicine. The plants have been used traditionally for centuries and modern scientific studies have shown the existence of good correlation between the traditional or folkloric application of some of the plants further strengthens the search for pharmacological active components from plants.[1]

The International Agency for Research on Cancer estimates of the incidence of mortality and prevalence from major types of cancer, at national level, for 184 countries of the world revealed that there were 14.1 million new cancer cases, 8.2 million cancer deaths, and 32.6 million people living with cancer (within 5 years of diagnosis) in 2012 worldwide [2]. By 2030, it is projected that there will be 26 million new cancer cases and 17 million cancer deaths per year. Today, despite considerable efforts, cancer still remains an aggressive killer worldwide. Moreover, during the last decade, novel synthetic chemotherapeutic agents currently in use clinically have not succeeded in fulfilling expectations despite the considerable cost of their development [3].

Therefore there is a constant demand to develop new, effective, and affordable anticancer drugs [4]. From the dawn of ancient medicine, chemical compounds derived from plants have been used to treat human diseases. Natural products have received increasing attention over the past 30 years for their potential as novel cancer preventive and therapeutic agents. In parallel, there is increasing evidence for the potential of plant-derived compounds as inhibitors of various stages of carcinogenesis and associated inflammatory processes, underlining the importance of these products in cancer prevention and therapy. Therefore, it is of interest to investigate the total content of various phytoconstituents of aqueous, methanol, and chloroform extracts of a poly herbal formulation of *Nalla marunthu* were screened.

MATERIALS AND METHODS

Collection of samples

The poly herbal formulation of *Nalla marunthu* is used for this study. The herbal formulation was prepared by the available literature.

Preparation of Herbal medicine

The herbal formulation *Nalla marunthu* was prepared in the department of Industrial Biotechnology, Bharath Institute of Higher Education and Research, Bharath University, Chennai, India. The equal volume of shade

dried leaves of *Indigofera tinctoria, A.polygonoides, T.portulacostrum and C.bonplandianus* were taken. The plant material was coarsely powdered, then filtered by muslin cloth and the filtrate was used for further extraction.

Preparation of extracts

1000 grams of *Nalla marunthu* was packed in three separate round bottom flask for sample extraction using solvents namely Aqueous, Chloroform and Methanol. The extraction was conducted by 250 ml of the each solvent mixture for a period of 24 hours. 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.

Quantification of Phytochemicals

Determination of total phenolic content, Determination of total tannin Content, Determination of total Alkaloid content and Determination of total flavonoid content were analysed [5-11].

Determination of total phenolic content

The concentration of phenolic components in plant extracts was determined using spectro photo metric method. Folin-Ciocalteu assay method was used for the determination of the total phenol content. The reaction mixture consists of 1 ml of extract and 9 ml of distilled water was taken in a volumetric flask (25 ml). One millilitre of Folin - Ciocalteu phenol reagent was treated to the mixture and shaken well. After 5 minutes, 10 ml of 7 % Sodium carbonate (Na_2CO_3) solution was treated to the mixture. The volume was made up to 25 ml. A set of standard solutions of gallic acid (20, 40, 40, 60, 80 and 100 µg/ml) were prepared in the same manner as described earlier. Incubated for 90 min at room temperature and the absorbance for test and standard solutions were determined against the reagent blank at 550 nm with an Ultraviolet (UV) /Visible spectrophotometer. Total phenol content was expressed as mg of GAE/gm of extract.

Determination of Tannin Content

The tannins were determined by Folin - Ciocalteu method. About 0.1 ml of the sample extract was added to a volumetric flask (10 ml) containing 7.5 ml of distilled water and 0.5 ml of Folin-Ciocalteu phenol reagent, 1 ml of 35 % Na2CO3 solution and dilute to 10 ml with distilled water. The mixture was shaken well and kept at room temperature for 30 min. A set of reference standard solutions of gallic acid (20, 40, 60, 80 and 100 µg/ml) were prepared in the same manner as described earlier. Absorbance for test and standard solutions were measured against the blank at 725 nm with an UV/Visible spectrophotometer. The tannin content was expressed in terms of mg of GAE /g of extract.

Determination of Alkaloids

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

Determination of Total flavonoid content

Total content of flavonoid was measured by the aluminium chloride colorimetric assay. The reaction mixture consists of 1 ml of extract and 4 ml of distilled water was taken in a 10 ml volumetric flask. To the flask, 0.30 ml of 5 % sodium nitrite was treated and after 5 minutes, 0.3 ml of 10 % aluminium chloride was mixed. After 5 minutes, 2 ml of 1M Sodium hydroxide was treated and diluted to 10 ml with distilled water. A set of reference standard solutions of quercetin (20, 40, 60, 80 and 100 µg/ml) were prepared in the same manner as described earlier. The absorbance for test and standard solutions were determined against the reagent blank at 510 nm with an UV/Visible spectrophotometer. The total flavonoid content was expressed as mg of QE/g of extract.

RESULTS AND DISCUSSION

Recent trends in drug development process are focused on natural sources, such as sources of plant origin due to some proven correlation between the folkloric medicinal uses of some of these plants to biological activity. Hence the use of plant materials to prevent and treat infectious diseases successfully over the years has continued to attract the attention of scientists worldwide [12,13].

The present study was performed to evaluate the total phenol, tannin, alkaloid and flavonoid contents in chloroform, ethyl acetate and methanol extracts of an herbal formulation *Nalla marunthu.* (Table 1, 2, 3 and 4, figure 1, 2, 3 and 4). Use of plants as a source of medicine has been inherited and is an important component of the health care system. India is the largest producer of medicinal herbs and is appropriately called the botanical garden of the world. Plants used for traditional medicine contain a wide range of substances that can be used to treat chronic as well as infectious diseases.[14]

The total phenolic compound ranged from 12.64 to 40.20. Total phenolic contents in the *Nalla Mrunthu* extract expressed in terms of gallic acid equivalent (mg of GAE/g of extract), (y = 0.549 x + 0.5479, R^2-0.9228). The total flavonoid content varied from 20.68 to 62.32. Concentrations of flavonoids in the poly herbal

formulation extract expressed in terms of quercetin equivalent (mg of Q E/g of extract) (y =1.3771 x +0.1, R^2-=1).The total content of alkaloids varied from 1.64 to 58.20 .

Table 1: Total phenolic content in the *Nalla marunthu* extract expressed in terms of gallic acid equivalent (mg of GAE/g of extract)

S.No	Extracts	mg of GAE/g of extract
1	Chloroformic extract	40.20 ± 0.016
2	Methanolic extract	12.64 ± 0.092
3	Ethyl acetate extract	22.48 ± 0.038

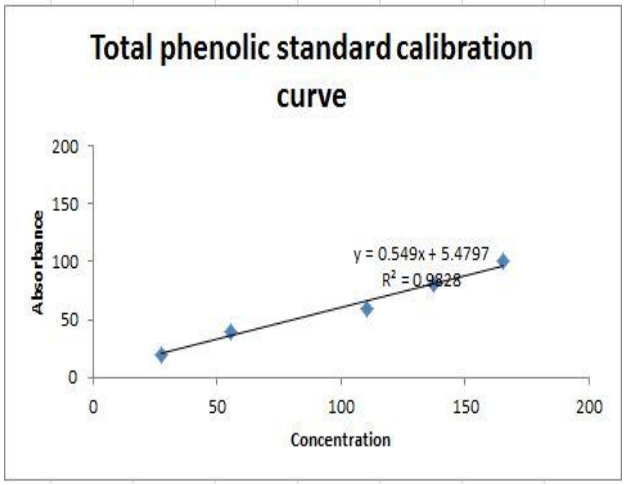

Figure 1: Calibration curve of total content of phenolic compounds.

Table 2: Alkaloid contents in the *Nalla marunthu* extract expressed in terms of atropine equivalent (mg of AE/g of extract)

S.No	Extracts	mg of AE/g of extrac
1	Chloroform extract	58.20 ± 0.640
2	Methanol extract	18.64 ± 0.012
3	Ethyl acetate extract	36.98 ± 0.064

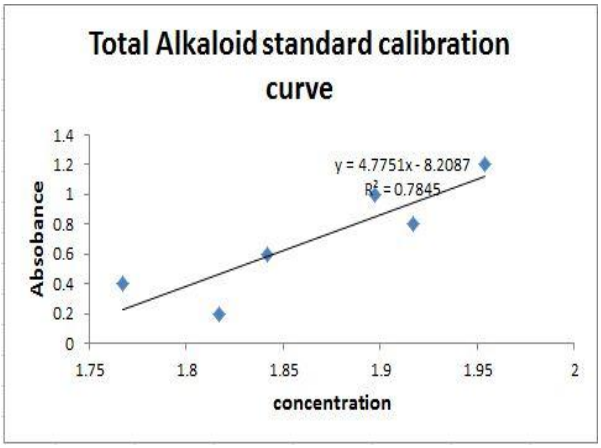

Figure 2: Calibration curve of total Alkaloid content

Table 3: Concentrations of flavonoids in the *Nalla marunthu* extract expressed in terms of quercetin equivalent (mg of QE/g of extract)

S.No	Extracts	mg of QE/g of extract
1	Chloroform extract	62.32 ± 0.087
2	Methanol extract	20.68 ± 0.052
3	Ethyl acetate extract	32.86 ± 0.048

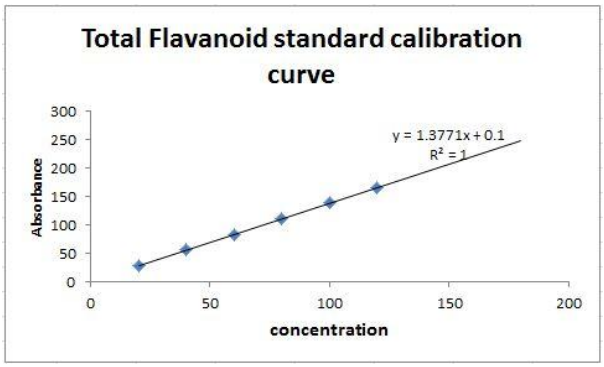

Figure 3: Calibration curve of total Flavonoid content

Table 4: Total Tannin contents in the *Nalla marunthu* extract expressed in terms of gallic acid equivalent (mg of GAE/g of extract).

S. No	Extracts	mg of GAE/g of extract
1	Chloroform extract	76.06± 0.096
2	Methanol extract	56.72± 0.062
3	Ethyl acetate extract	28.12 ± 0.014

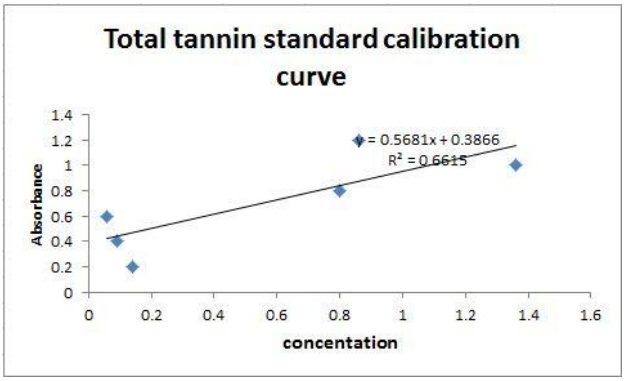

Figure 4: Shows the Calibration curve of total Tannin content

Alkaloid contents in the herbal formulation expressed in terms of atropine equivalent (mg of AE/g of extract) (y =4.7751 x +8.2087, R^2 -0.7846). The total content of tannins varied from 28.12 to 76.06. Tannin contents in the poly herbal formulation extract expressed in terms of gallic acid equivalent (mg of GAE/g of extract. (y =0.5681 x +0.3865, R^2-0.6619). The pharmacological action of chloroformic extract of *Nalla marunthu* was determined by the nature of these chemical compounds which are responsible for the desired therapeutic properties and definite physiological effects [15].

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