Research Article

Quantitative Analysis of Phytoconstituents of Chloroform Extract of Poly Herbal Formulation

S.Selva Kumar *, U.Madhan Kumar, A. Prashanth, Barnali Sarkar, J. Sindhuja
Department of Industrial BioTechnology, Bharath University, Chennai, India.
***Corresponding author's E-mail:** selvakumarmss@gmail.com

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ABSTRACT

The current mode of treatment for various diseases is based on synthetic drugs. These drugs are effective but they show serious adverse effects and also alter the genetic and metabolic activity of the patient. More over some drugs prepared from medicinal plants and their constituents show more efficacy than the synthetic counterparts. Earlier reports have shown that the regular consumption of herbs, fruits and vegetables are strongly related with reduced risk of various forms of diseases. Therefore, it is of interest to investigate the quantification of phytoconstituents such as total content of poly phenols, alkaloids, flavonoids and total content of tannins of chloroformic extract of herbal formulation were undertaken. Our results indicate that the presence of various phytocomponents in the poly herbal formulation.

Keywords: Alkaloids, Flavonoids, Tannins, Poly phenols, Chloroform, Phytocompounds.

INTRODUCTION

Plants have provided mankind with herbal remedies for several diseases for many centuries. In India herbal medicines have been the bases of treatment and cure for various diseases in traditional methods such as Ayurveda, Unani and Sidha. The therapeutic potentials of plant and animal origin crude drugs are being used from the ancient times by the simple process without the isolation of the pure compounds. The pharmacological action of crude drug is determined by the nature of its constituents. Thus the plant species may be consider as a biosynthetic and for the chemical compounds example proteins, carbohydrates, and fats that are utilized as food by the animals and humans, but also for a huge number of compounds including alkaloids, terpenoids, flavonoids, glycosides etc. which exert definite physiological effects. These chemical compounds are mostly responsible for the desired beneficial properties[1].

Trianthema portulacastrum Linn. is a herb used in Ayurvedic medicine. T*rianthema portulacastrum* Linn, belonging to the family Aizoaceae, is one of the common weed, which has enormous traditional uses against diseases and some bioactive compounds have been isolated from this weed. It is an exotic weed and a native of tropical America. It is growing throughout most tropical countries, such as Baluchistan, Ceylon, and India [2]. It is .now naturalized throughout India in cultivated fields, river beds, waste ground, etc [3]. Its infestation is very common in various agricultural and vegetable crops, such as mustard, maize, pigeon pea, mung bean, potato, onion, cotton, soybean, pearl millet, and sugarcane, especially during the rainy seasons. This is not cultivated commercially, but it is found throughout India as a tropical problematic terrestrial weed by virtue of its infestation in plains, river beds, and in wastelands. It also grows automatically in cultivated fields with agriculture and vegetable crops; especially in the rainy seasons[4]. *Amaranthus polygonoides* is a herb the pharmacological properties of Amaranth products are considered of vital importance. For reducing tissue swelling the leaves are well thought-out to be constructive, and they have a cleansing effect too. The plant has also been used curatively for diarrhea, dysentery, excessive menstrual flow, ulcers and intestinal hemorrhaging. For the treatment of intestinal bleeding, excessive menstruation, diarrhea and other related problems, a tea made from its leaves are used [5].

The plant *Indigofera tinctoria* belongs to the family of Fabaceae, its commonly known as "True Indigo'' and called as Neeli or Avuri in Tamil. *I. tinctoria* is a shrub, distributed throughout the India. The different parts of the plants are used for variety of diseases. The seeds of *I. tinctoria* containing galactomannon composed of galactose and mannose. Roots and leaves are used in epilepsy and hydrophophia. The aerial parts of *I. tinctoria* used in treatment of anti proliferative activity in human lung cancer[6]. Different solvent extracts of *I. tinctoria* showed antibacterial activity [7]. Dry powder of *I. tinctoria* used to treatment of asthma[8]. The leaves of *I. tinctoria* used as anti inflammatory traditionally. Indirubin is the active compounds isolated from *I. tinctoria* leaves active as effective anticancer drug Indigotin is the active compounds isolated from this leaves possess hepatoprotective activity[9, 10].

MATERIALS AND METHODS

Collection of samples

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

Preparation of Herbal medicine

The herbal formulation 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 *Hugonia syntax*, W*edelia trilobata* and *Cassia alata* were taken in to mortar and pistle. 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 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 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 [10-22].

Determination of total phenolic content

The concentration of phenolics in plant extracts was determined using spectrophotometric 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 milliliter 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 dimethyl sulphoxide (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 content of flavonoids

Total content of flavonoid was measured by the aluminum 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 % aluminum 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

Quantification of herbal formulation

The present study was performed to evaluate the total content of phenol, tannin, alkaloid and flavonoid in aqueous, chloroform, ethyl acetate and butyl alcohol extracts of an herbal formulation. Extraction of herbal powder material was carried out by continuous hot percolation method in soxhlet apparatus using aqueous, chloroform, ethyl acetate and butyl alcohol as solvents (Figure1 - 8) Gallic acid was used as standard for the determination of total phenol and tannin by Folin - ciocalteu method.

Total alkaloid content was determined by colorimetric method using quercetin as a standard and Total phenol content was expressed as mg of GAE/gm of extract($y=388.5\times103.7$ and $R^2=0.617$). The results showed that the Chloroformic extract has high concentration of total phenol, tannin, alkaloid and flavonoid content as compared by bromocresol green solution using atropine as a standard. Total alkaloid contents in the herbal formulation extract expressed in terms of atropine equivalent (mg of AE/g of extract) ($y=0.663\times0.056$, $R^2=0.959$).

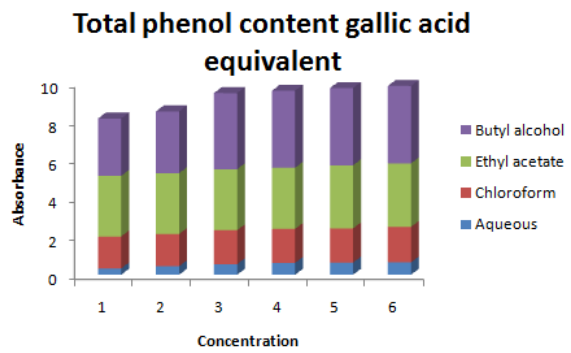

Figure-1: Total phenolic contents in the herbal formulation extract expressed in terms of gallic acid equivalent (mg of GAE/g of extract).

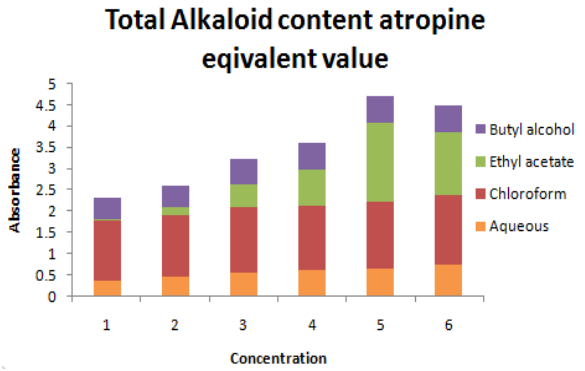

Figure 2: Total alkaloid contents in the herbal formulation extract expressed in terms of atropine equivalent (mg of AE/g of extract).

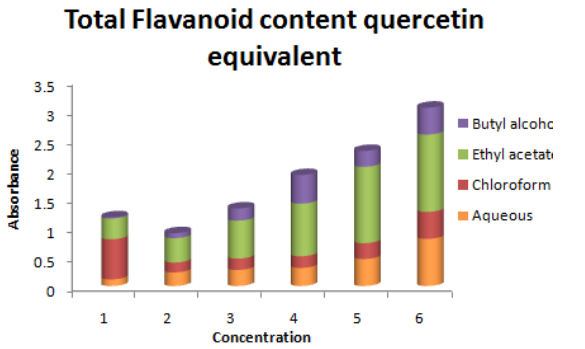

Figure 3: Total flavonoid content in the herbal formulation extract expressed in terms of atropine equivalent (mg of AE/g of extract).

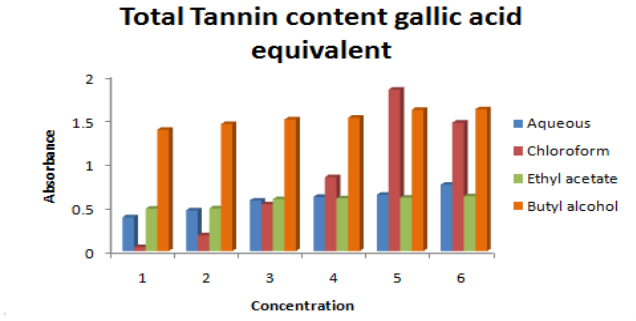

Figure 4: Total Tannin contents in the herbal formulation extract expressed in terms of gallic acid equivalent (mg of GAE/g of extract) (y =145.9×0.224, R^2=0.938).

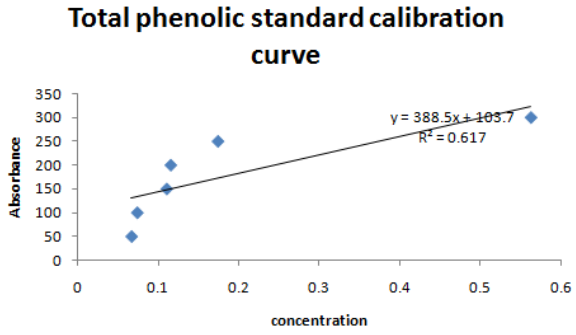

Figure 5: Calibration curve of total phenolic content.

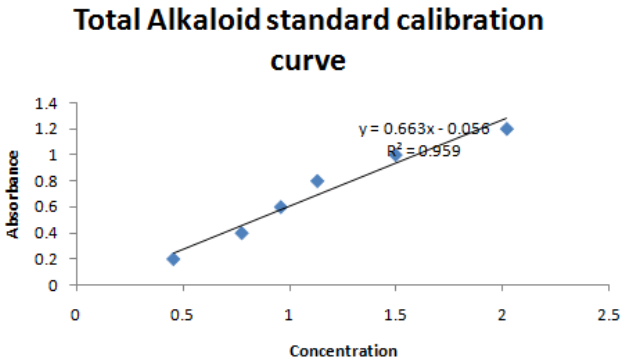

Figure 6: Calibration curve of total alkaloid content.

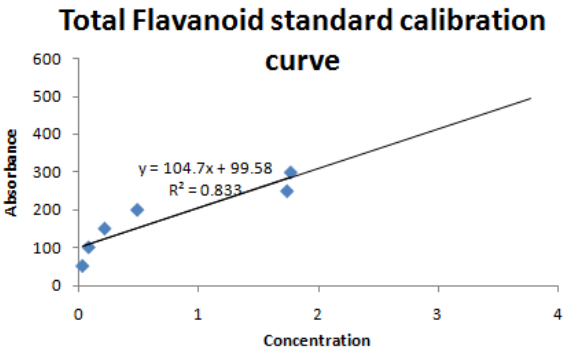

Figure 7: Calibration curve of total flavonoid content

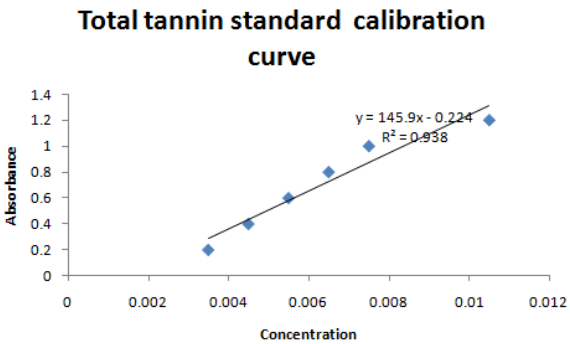

Figure 8: Calibration curve of total Tannin content.

Total flavonoid content in the herbal formulation extract expressed in terms of atropine equivalent (mg of AE/g of extract)(y=104.7×99.58, R^2 =0.883). The pharmacological action of chloroform extract of herbal formulation were determined by the nature of these chemical compounds which are responsible for the desired therapeutic properties and definite physiological effects. All the calibration graphs showed that strong positive linear

correlation (r) which is close to +1. The graphs indicate that as the value of concentration increases, values for absorbance also increase. The total phenol, alkaloid, flavonoid and tannin contents are more in chloroform extract when compared to aqueous, ethyl acetate and butyl alcohol so the quantification of four solvents has been done with the different concentrations in the required methods. The concentration of flavonoids in plant extracts depends on the polarity of solvents used in the extract preparation. The different standard calibration curves was established between phenol, alkaloid flavonoid and tannin contents. The standard sample values were observed with the different calibration curves and the different concentrated values was obtained with the help of different graphs. So from the following above one comparing to all the solvents aqueous, ethyl acetate and butyl alcohol the chloroformic extract yields highest concentrations of phenols, alkaloids, flavonoids and tannins so further different pharmacological activities can be carried with the help of these chloroform solvent.

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