ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Phytochemical Quantification of Chloroform Extracts of *Ctenolepsis cerasiformis.*

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ABSTRACT

Ctenolepsis cerasiformis is a medicinal plant distributed in south India and belongs to the family of cucurbitaceae and spreading on low shrubs or climbing; stem subfiliform, elongate, much branched, grooved and angled, glabrous except the sparse hairs at the node. Tendrils slender, elongated, simple. Leaves 3.5-10 cm long and almost equally broad, lamina broadly ovate-cordate in outline, scabrid-punctate above and beneath, scab rid hairs pointing forward on nerves and margins, palmate 3-(rarely 5-)-lobed, segments usually ovate-oblong, acute, narrow at the base, the lateral segments often with apparent macro. Petiole 2-4 cm long; stipule bracts 7-15 mm long, more or less sub orbicular, ciliate with hairs as long as the breadth of bract. Flowers minute (petals spreading, ovate-ligulae, almost free, 1.5 mm long, 1 mm broad); male flowers 5-10 at the apex of 2-4 cm long peduncles, pedicel bracteates, 2-3 mm long; female flowers solitary on short peduncles; ovary globosely, slightly beaked, 2 mm long, 1.5 mm across. Fruit globosely or oblate, glabrous and 1.3 cm in diameter. Seeds 2, ovoid 8 mm long, 5 mm broad, ovate-perform, Plano-convex, not bordered, smooth, edges compressed. Hence, it is of interest to investigate the phytochemical profile of chloroform extract of Ctenolepsis cerasiformis were undertaken. Our results indicate that the presence of various Phytochemical.

Keywords: Ctenolepsis cerasiformis, Polyphones, Tannins, Alkaloids, Flavonoids, Phytochemical quantification.

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ISSN: 0975-8585

INTRODUCTION

Flavonoids are the polyphenolic phytochemical with inconsistent Phenolic structures and they consist of flavones, flavanone, flavones, flavones and flavanonols that comprise a large group of secondary metabolites in plants [1]. Flavonoids are found in vegetables, fruits, flowers, grains, barks, roots and stems [2]. They have significant bio–pharmacological activities such as anti microbial, antioxidant, anti cancer and anti-inflammatory activity [3-4]. Present study was carried out to investigate the total content of flavonoids in the chloroform extract of Ctenolepsis cerasiformis due to its significance in curing different human diseases.

Alkaloids is refers to a class of nitrogen containing organic compounds in the Plantae, most of them have more complex cyclic structure, the nitrogen atom is combined with inside the loop, mostly appears alkaline, and combines with acid to be salt; many of them have significant physiological activity. In plants, a few of very weak alkaline alkaloids present in free form, such as an amide alkaloids. Alkaloids which have an alkaline mostly exist in organic salts form, such as citrate, oxalate, tartrate, succinate and so on. Few exist in inorganic salt form, such as berberine, such as morphine sulfate. There are still other forms of N-oxide alkaloid glycosides. Most plants containing alkaloids are a variety of alkaloids coexist and alkaloid biosynthesis pathway are often similar in the same plant, so chemical structure is also similar, belong to the same family of plants often have the same parent nucleus, or the same structure as the compound [5].

Tannins are polymeric Phenolic compounds with numerous hydroxyl groups and quite diverse in chemical structure. Hydrolysis of some of tannins yields the simple, seven-carbon Gallic acid; others give ellagic acid or other Phenolic acids. Tannins are generally divided into the hydrolysable and condensed tannins. Tannins are “water-soluble Phenolic compounds having molecular weight between 500 and 3000, and besides giving the usual Phenolic reactions, they have special properties such as the ability to precipitate alkaloids, gelatin and other proteins [6]. Tannins are divided into three main classes. The condensed tannins (proanthocyanidins) are flavan-3-ol based biopolymers that at high temperature in alcohol solutions of strong mineral acid release anthocyanidins and catechins as end groups. Gallotannins and ellagitannins belong to hydrolysable tannins. Gallotaninns are comprised of galloyl esters of glucose or quinic acid whereas ellagitannins are derivatives of hexahydroxydiphenic acid [7]. Tannins act as an anti nutrient compound of plant origin because they precipitate proteins, inhibit digestive enzyme, and decrease the utilization of vitamins and minerals. Yet, tannins have also been considered a health-promoting component in plant-derived foods and beverages. For example, tannins have been shown to have anti carcinogenic and anti mutagenic potential, and antimicrobial properties. Several studies have reported on antioxidant and antiradical activity of tannins [8].

Polyphones fall into many different families including anthocyanins, coumarins, lignins, flavonoids, tannins, quinones, acids and phenols. This structural diversity results in large variability of the physic-chemical properties influencing the extraction of polyphenols6. Distribution of phenolics in plants at the tissue, cellular and sub cellular levels is not always in similar. Soluble phenolics are present within the plant cell vacuoles, insoluble phenolics are found in cell walls but the outer layers of plants contain higher levels of phenolics than those located in their inner parts. Polyphenolic content of the foods is greatly affected by environmental edaphic factors like soil type, sun exposure and rainfall etc. Another factor that directly affects the polyphenol content of the foods is storage. Stored foods show change in polyphenolic content due to easy oxidation of these polyphenols. Oxidation reactions result in the formation of more or less polymerized substances resulting in change in food quality, colour and organoleptic characteristics. Such changes may be beneficial, as is the case of black tea7. To avoid degradation of native polyphenols, samples are often dried, frozen or lyophilized before extraction because high moisture or water content aids the activity of enzymes [9].

MATERIALS AND METHODS

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 Phytochemical

Determination of total Phenolic content, Determination of total tannin Content, Determination of total Alkaloid content and Determination of total flavonoids content were analyzed [10-22].

Determination of Total content of Alkaloids

The plant extract (1mg) was dissolved in dimethyl sulphoxide (DMSO), added 1ml of 2N 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 flavanoids

Total content of flavanoid 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 % 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 flavanoid content was expressed as mg of QE/g of extract.

Determination of Total content of Tannins

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 % Na_2CO_3 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 total content of Phenolic compounds

The concentration of phenolics in plant extracts was determined using spectrophotometric method. FolinCiocalteu 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 Folic -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.

ISSN: 0975-8585

RESULTS AND DISCUSSION

Figure 1: The Standard Calibration curve of Alkaloids.

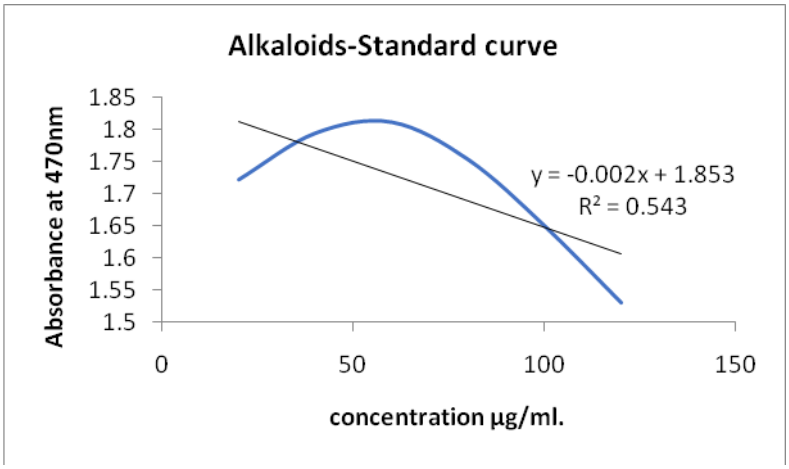


Figure 2: Alkaloid contents in the Ctenolepsis cerasiformis extract expressed in terms of atropine equivalent (Mg of AE/g of extract)

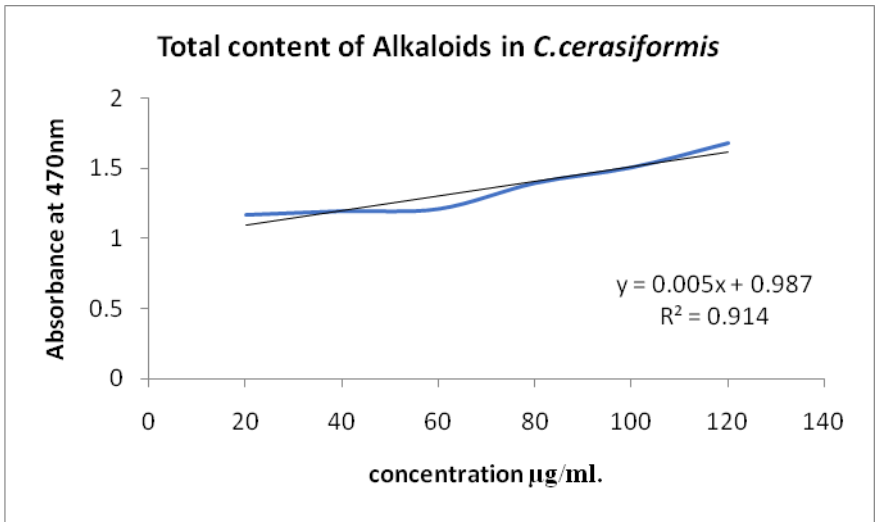
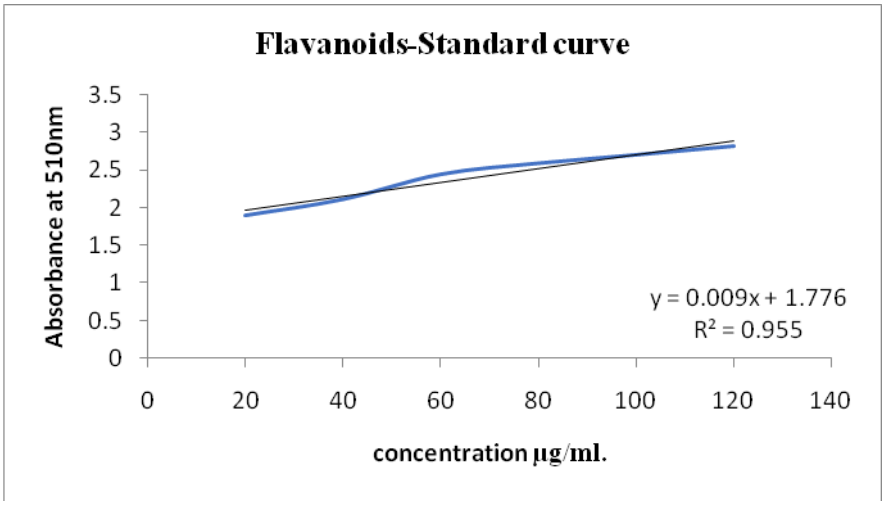


Figure 3: Standard Calibration Curve of Flavonoids.



ISSN: 0975-8585

Figure: 4 Concentrations of flavonoids in the chloroform extract of Ctenolepsis cerasiformis expressed in terms of quercetin equivalent (mg of QE/g of extract).

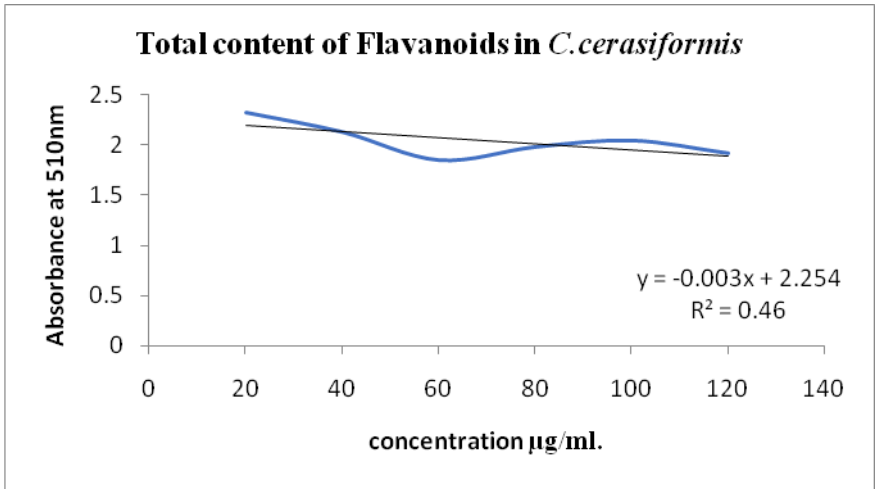


Figure 5: Standard Calibration curve of Tannins.

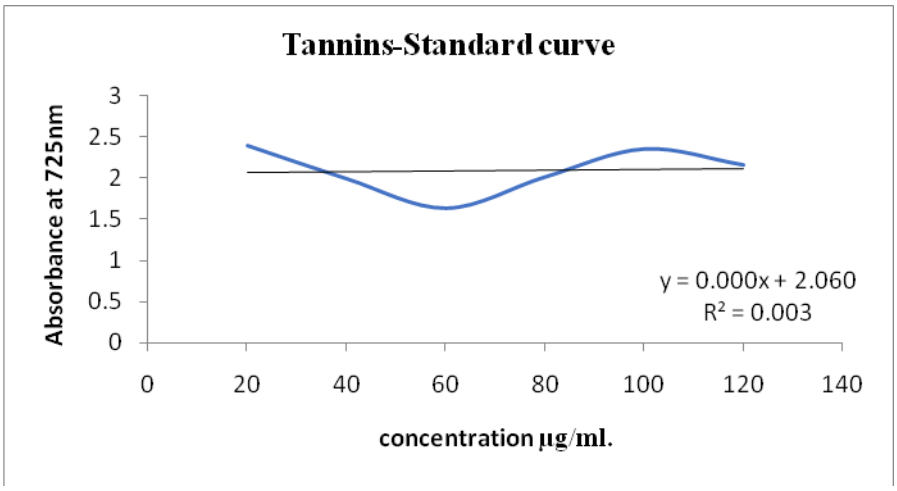
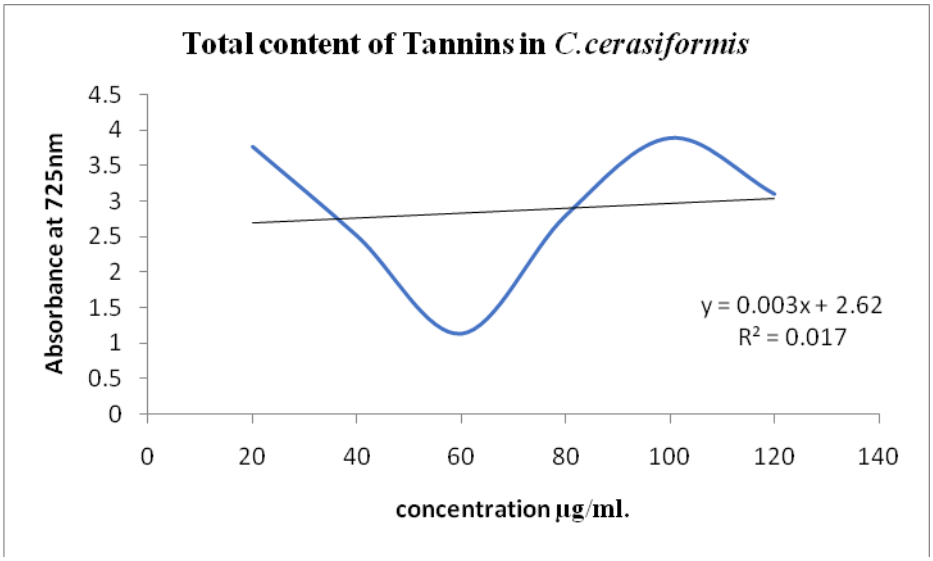


Figure 6: Total Tannin contents in the C.cerasiformis extract expressed in terms of Gallic acid equivalent (mg of GAE/g of extract).



ISSN: 0975-8585

Figure: 7: Standard Calibration Curve of Phenolic compounds.

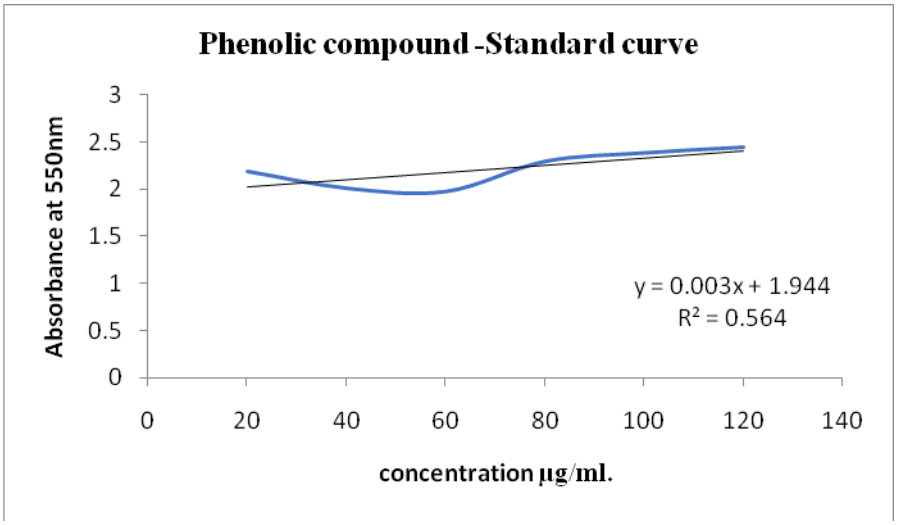
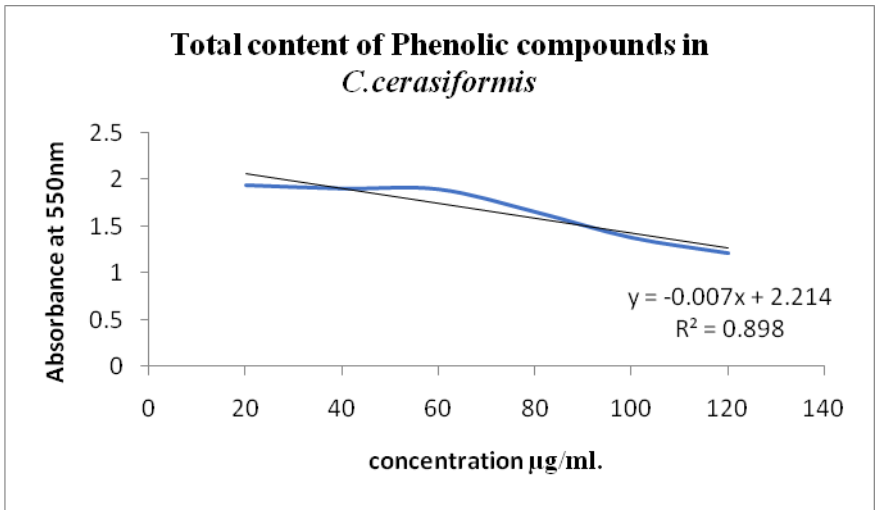


Figure 8: Total Phenolic content in the Ctenolepsis cerasiformis extract expressed in terms of Gallic acid equivalent (mg of GAE/g of extract).



The present study was performed to evaluate the total phenol, tannin, alkaloid and flavonoids contents in chloroform extract of Ctenolepsis cerasiformis (figure 1-8). Figure 1 and 2 shows that the total alkaloids content in standard and chloroform of extract of Ctenolepesis cerasiformis. Total alkaloids content in the extract expressed in terms of atropine equivalent (mg of AE/g of extract) (y =0.002x +1.853, R^2=0.543), (y=0.005x+0.987, R^2=0.914). Figure 3 and 4 shows that the total flavonoids content in standard and chloroform of extract of Ctenolepsis cerasiformis. Total flavonoids content in the extract expressed in terms of quercetin equivalent (mg of Q E/g of extract) (y= 0.009x+ 1.776, R^2= 0.955), (y= 0.003x+ 2.254, R^2= 0.46). Figure 5 and 6 shows that the total tannins content in standard and chloroform of extract of Ctenolepsis cerasiformis. Total Tannins content in the extract expressed in terms of Gallic acid equivalent (mg of GAE/g of extract. (y =0.000x +2.060, R^2=0.003), (y=0.003x+2.62, R^2=0.017). Figure 7 and 8 shows that the total Phenolic compound content in standard and chloroform of extract of C.cerasiformis. Total Phenolic contents in the extract expressed in terms of Gallic acid equivalent (mg of GAE/g of extract) (y=0.003x+1.944, R^2=0.564), (y= 0.007x+2.214, R^2 =0.898). The Pharmacological, Toxicological and Biochemical mechanism of action of chloroformed extract of Ctenolepsis cerasiformis was determined by the nature of these phytocompounds which are responsible for the desired therapeutic properties and definite physiological effects [18].

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