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Determination of Total Phenol, Alkaloid, Flavonoid and Tannin in Different Extracts of *Calanthe Triplicata.*

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Research Article

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Keywords: Phenol, flavonoid, alkaloid, tannin.

ABSTRACT

The present work was aimed to evaluate the total phenol, alkaloid, flavonoid and tannin contents in petroleum ether, chloroform, ethyl acetate and methanol extracts of *Calanthe triplicata* (Williemet) Ames. Gallic acid was used as standard for the determination of total phenol and tannin by Folin-Ciocalteu method. Total alkaloid content was determined chloride colorimetric method using quercetin as a standard. The results showed that ethyl acetate extract has high concentration of phenol, alkaloid, flavonoid and tannin contents as compared by bromocresol green solution using atropine as a standard. Total flavonoid content was determined by aluminium to petroleum ether, chloroform, ethyl acetate and methanol extracts. Ethyl acetate extract contained the total phenol of 29.43 and tannins of 82.92 as mg of gallic acid equivalents (GAE), alkaloids of 65.34 as mg of atropine equivalents (AE) and flavonoids of 90.24 as mg of quercetin equivalents (QE).

INTRODUCTION

The therapeutic potentials of plant and animal origin 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]. *Calanthe triplicata* is a species of orchid from the genus *Calanthe* and belongs to the family of Orchidaceae. This plant is a low growing evergreen terrestrial orchid, snow white flowers with yellow or red callus found in the hilly parts of south India. The herbs are reported to be used in diseases of stomach and intestine; and the root is chewed along with betel nuts or other aromatic substances, in diarrhea [2].

MATERIALS AND METHODS

Chemicals and reagents

Sodium carbonate, aluminium chloride, sodium nitrite, sodium hydroxide was obtained from Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Sri Ramachandra University. Folin-Ciocalteu's phenol reagent, aluminium chloride, gallic acid and quercetin were obtained from Department of pharmacognosy, Faculty of Pharmacy, Sri Ramachandra University.

Plant material

The plant was collected from Kolli hills, Namakkal district, Tamil Nadu and authenticated by Dr. Sasikala Ethirajulu, Assistant Director (Pharmacognosy), Siddha Central Research Institute Arignar Anna Govt. Hospital Campus Arumbakkam, Chennai, Tamil Nadu, India.

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Preparation of Extracts

Freshly collected plants were dried in shade and finely powdered. Then the powdered plant material was extracted successively with petroleum ether, chloroform, ethyl acetate and methanol by cold maceration method. The solvent was removed by distillation. Then the extracts were evaporated and concentrated [3].

Determination of total phenolic content

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/g of extract [4-6].

Determination of Alkaloid

The plant extract (1mg) was dissolved in dimethyl sulphoxide (DMSO), added 1ml of 2 N HCl 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 [7, 8].

Determination of Total flavonoid content

Total flavonoid content 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 [9-12].

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 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 [13,14,15,16].

RESULTS AND DISCUSSION

Total phenol, alkaloid, flavonoid and tannin contents were calculated by using calibration curve. The results are tabulated in Table 1 and calibration curve for total phenol, alkaloid, flavonoid and tannin contents were shown in Figure 1, 2, 3 and 4respectively.

Table 1: Total phenol, alkaloid, flavonoid and tannin contents

Phytochemical Content	Petroleum ether	Chloroform	Ethyl acetate Extract	Methanolic Extract
Total Phenol Content (mg of GAE/g of extract)	12.3± 0.31	18.67±0.21	29.43±0.31	23.53±0.25
Alkaloid Content mg of AE / g of extract)	18.7± 0.2	21.77±0.33	65.34±0.05	40.85±0.03
Total Flavonoid Content (mg of QE/g of extract	61.34±0.05	57.70±0.02	90.24±0.04	65.94±0.03
Tannin Content(mg of GAE/g of extract)	45.28±0.06	38.93±0.05	82.92±0.03	55.09±0.02

Values are reported as mean of three evaluations ± Standard deviation

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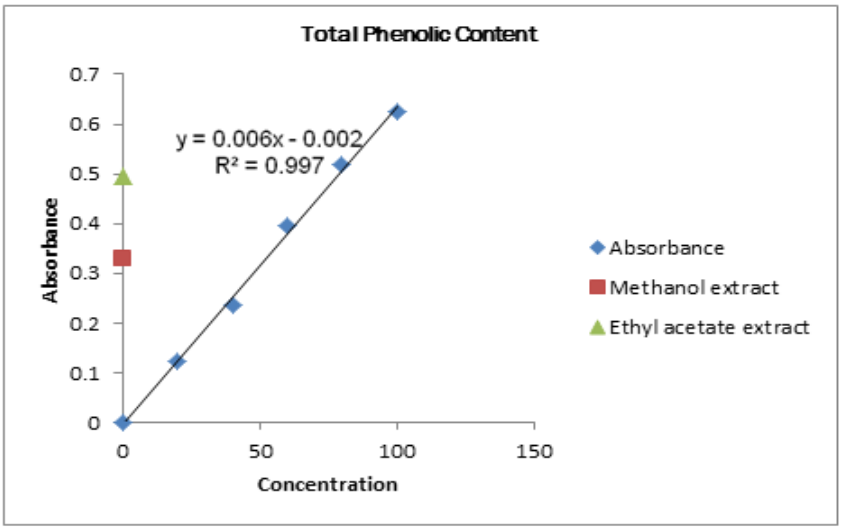

Figure 1: Total Phenolic Content

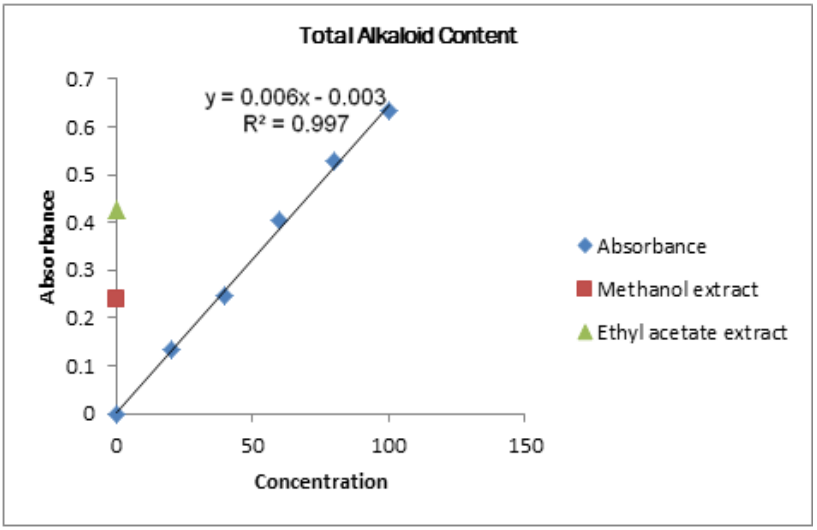

Figure 2: Total alkaloid content

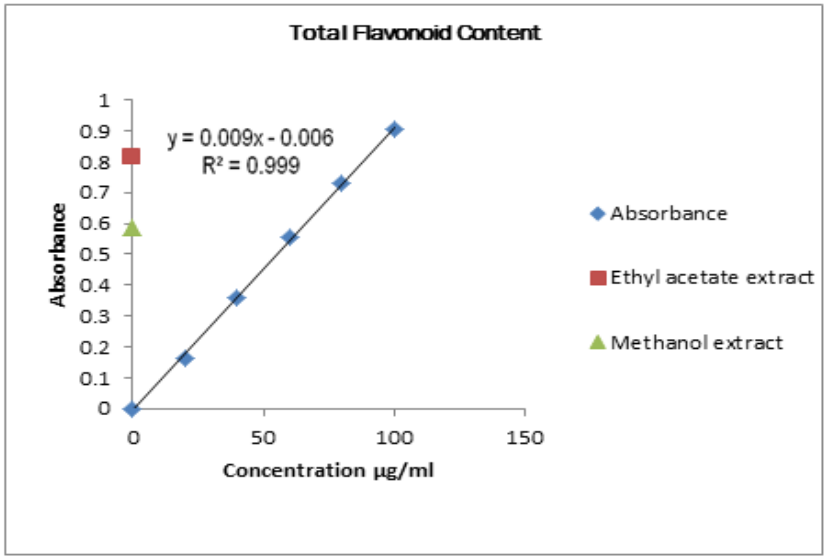

Figure 3: Total flavonoid content

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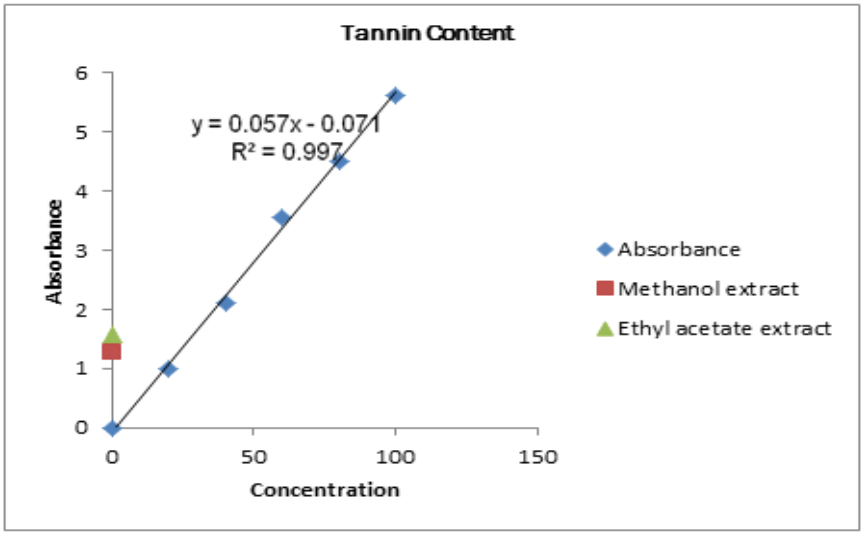

Figure 4: Tannin Content

All the calibration graphs showed that strong positive linear correlation (r) which is close to +1. These graphs indicate that as the value of concentration increases, values for absorbance also increase. Total phenol, alkaloid, flavonoid and tannin contents in ethyl acetate extract were found to be 29.43 mg of GAE, 65.34 mg of AE, 90.24 mg of QE and 82.92 mg of GAE/gm of extract respectively. Petroleum ether, chloroform and methanolic extracts were made known less content of phenol, alkaloid, flavonoid and tannin.

CONCLUSIONS

In this study, total phenol, alkaloid, flavonoid and tannin contents from the whole plant of ethyl acetate and methanolic extracts of *Calanthe triplicata* were investigated. Ethyl acetate extract showed high contents of phenol, alkaloid, flavonoid and tannin as compared to methanolic extract. The pharmacological action of ethyl acetate extract of *Calanthe triplicata* will be determined by the nature of these chemical compounds which are responsible for the desired therapeutic properties and definite physiological effects.

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