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Quantitative Standardization Of Different Extracts Of Grewia Tiliaefolia Vahl Leaf

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Abstract

The medicinal qualities of plants are of course due to phytoconstituents. The current study evaluates the total phenolic, flavonoid, alkaloid content and the concentration of the total phenolic, flavonoid, and alkaloid content in successively extracted hexane, ethyl acetate, methanolic extracts was determined using spectrophotometric methods. The total phenolic 22.79±0.23,55.32±0.10, 87.58±0.25 (mg GAE/g dried extract), total flavonoid 10.24±0.98,17.4±0.32, 21.47±0.42 (mg QE/g dried extract) and total alkaloid content in plant extracts 14.86±0.77, 13.44±0.42, 18.2±0.35 (mg A/g dried extract) respectively.

Keywords: Phenolic, Flavonoid, Alkaloid Content, Grewia tiliaefolia Vahl, Gallic acid, Quercetin, Atropin

Introduction

In recent years, herbal medicines have been highlighted for their contribution towards lowering the risks of several life-threatening diseases such as coronary heart disease, stroke, pulmonary disease, and different types of cancer [1]. The benefits are due to the presence of polyphenols, flavonoids, carotenoids, and vitamins. Of these phytochemicals, alkaloids, Phenols, flavonoids have been reported on their effective antioxidants, anticancer, antibacterial, cardioprotective agents, anti-inflammation, immune system promoting, skin protection from UV radiation, and interesting candidate for pharmaceutical and medical application [2-7]. Since a few decades ago, the research studies focusing on flavonoids and the other phenolic compounds from medicinal plant species have increased considerably, because of their versatile benefits for human health [8-12]. Most of the recent reviews focused on one precise aspect of flavonoids or phenolics' action on human health. Natural products, such as plant extracts, either as pure compounds or as standardized extracts, provide unlimited opportunities for new drug discoveries because of the unmatched availability of chemical diversity (Cos et al., 2006). The current investigation is about the yield of the extract obtained, qualitative analysis, and quantitative estimation of total phenolic, flavonoid, and alkaloid content different extracts of Grewia tiliaefolia Vahl leaf.

Materials and Methods

Sodium dihydrogen phosphate, Disodium hydrogen phosphate, Potassium ferricyanide, Ferric chloride, Hydrochloric corrosive, Sodium hydroxide Folin-Ciocalteu reagent, gallic corrosive, and quercetin, atropin gauges Aluminum chloride hexahydrate, methanol, and sodium carbonate were obtained from Sigma Chemical Company, St.Louis, USA, and S.D.Fine Chemicals.

Instruments

Rotavap (Buchi Labortechnik AG, CH-929 Flawil 1, Switzerland), Vacuum Pump (Millipore) Vaccum PR, Pump 4 BAR, Uv-Visible Spectrophotometer (Thermo Scientific UV-10)

Preparation of plant extract

Freshly collected leaves were washed with tap water for the removal of earthy matter and dirt. They were shade dried and coarsely powdered using a Wiley mill. The powdered material was successively extracted with hexane and the same plant material re-extracted with ethyl acetate, methanol after complete drying of solvent using soxhlet apparatus and a fresh plant material extracted using methanol to get a crude methanolic extract and a fresh plant material extracted with distilled water to get crude aqueous extract by boiling the plant material with distilled water. The liquid fractions were filtered using a vacuum pump using Whatman filter paper No.1 and then the filtrate collected was evaporated under reduced pressure using a rotary evaporator (Buchi 210) at a temperature of 45^0c until a soft mass was obtained which is collected in a china dish and placed on a water bath for the removal of excess of solvent. The extract which is completely free from solvent was weighed and stored in a desiccator and used for further investigation.

Preparation of standard Gallic acid solution

1mg/mL of gallic acid standard stock solution was prepared in methanol from that different concentration 15, 25, 50, 75, 100 µg/mL was prepared. The reaction mixture was prepared by mixing 0.5 ml of different concentrations, 2.5 mL of 10% Folin-Ciocalteu's reagent, and 2.5 mL 7.5% Na HCO3. Blank was concomitantly prepared, containing 0.5 ml methanol, 2.5 ml 10% Folin- Ciocalteu's reagent, and 2.5 mL of 7.5% of NaHCO3. The samples were thereafter incubated in a thermostat at 45 C for 45 min. The absorbance was determined using a spectrophotometer at λmax, 765 nm.

Evaluation of Total Phenolic content

The concentration of phenolics in plant concentrates was resolved to utilize the spectrophotometric strategy [13]. The methanolic solution of the concentrate in the concentration of 1 mg/ml was utilized in the investigation. The response blend was set up by blending 0.5 ml of methanolic solution of concentrate, 2.5 ml of 10% Folin-Ciocalteu's reagent, and 2.5 ml 7.5% $NaHCO_3$. Blank was concomitantly prepared, containing 0.5 ml methanol, 2.5 ml 10% Folin-Ciocalteu's reagent, and 2.5 ml of 7.5% of NaHCO3. The samples were thereafter incubated in a thermostat at 45 C for 45 min. The absorbance was determined by utilizing a spectrophotometer at λmax = 765 nm. The samples were set up in triplicate for every examination and the mean estimation of absorbance was obtained. A similar technique was repeated for

the standard solution of gallic acid and the calibration line was construed. Based on the measured absorbance, the concentration of phenolics was read (mg/ml) from the adjustment line; at that point, the substance of phenolics in concentrates was communicated as far as Gallic corrosive proportionate (mg of GA/g of concentrate).

Preparation of standard curve of Quercetin

The aluminum chloride method ($AlCl_3$) was used to determine the total flavonoid content [14]. The different concentrations of Quercetin (20, 40, 60, 80, 100 µg) were pipetted with the help of a micropipette from 1mg/ml solution of Quercetin in Methanol and was used as standard. 0.3 ml of Sodium Nitrite (5 % w/v) was added and after 5 minutes 0.3 ml Aluminium Chloride (10 %w/v) was added and 2.0 ml sodium hydroxide (1 M) was added and volume made up to 10 mL with distilled water. The absorbance was read thereafter at 510 nm using water as blank.

Quantification of Total flavonoid content

The aluminum chloride technique was utilized to decide the absolute flavonoid content [10]. The various groupings of Quercetin (20, 40, 60, 80, 100, 8) µg was pipetted with the assistance of micropipette from 1mg/ml arrangement of Quercetin in Methanol and was utilized as standard. 0.3 ml of Sodium Nitrite (5% w/v) was included and 5 minutes later 0.3 ml Aluminum Chloride (10%w/v) was included and 2.0 ml sodium hydroxide (1 M) was added and volume made up to 10.0 ml with distilled water. The absorbance was read thereafter at 510 nm using water as blank. Quantification of total flavonoid content 0.1 ml of extract was added to 0.3 ml of 5% w/v Sodium nitrite solution. Kept it at room temperature for 5 minutes and 0.3 ml 10 % w/v Aluminum chloride was added. At that point following a moment included 2.0 ml of Sodium hydroxide arrangement (1N) and volume made up to 5ml with distilled water. After 10 min of incubation at ambient temperature, the absorbance was measured at 415 nm by using a UV-VISIBLE spectrophotometer. The total flavonoid contents were expressed as Quercetin equivalence (QE) in mg/g of sample.

Preparation of standard curve of Atropine

1.002 g of atropine was weighed and dissolved in100 mL volumetric flask with distilled water to make up the concentration. From the stock solution, five concentrations of 20 to 100µg/ml were prepared.

Quantification of Total Alkaloid content

The plant extracts (1mg) were dissolved in dimethyl sulphoxide (DMSO) .1ml of 2 N HCl was added and filtered. This filtrate was transferred to a separating funnel, 5 ml of bromocresol green solution and 5ml of phosphate buffer were added. The mixture was shaken with 1, 2, 3, and 4ml chloroform by vigorous shaking and collected in a 10ml volumetric flask and diluted to the volume with chloroform. The absorbance for test and standard solutions were determined against the reagent blank at 470 nm with a UV/Visible spectrophotometer. The total alkaloid content was expressed as mg of AE/g of the extract [15]. The results are expressed as atropine equivalents (mg of A/ g dry extract).

Results and Discussion

The total phenolic contents in the examined plant extracts using the Folin-Ciocalteu's reagent is expressed in terms of gallic acid equivalent (the standard curve equation: y = 0.004x - 0.015, r2 = 0.998). The values obtained for the concentration of total phenols are expressed as mg of GA/g of extract (Table 1). Differences were observed for total phenolic contents among the extracts. TPC was in the range of 22.79-87.58 mg GAE/g dried extract. The highest total phenolic contents were observed in methanolic extract followed by ethyl acetate, and hexane, extracts. Generally, the phenolic content of all the extracts was considerably high, which could be a major contributing factor to the strong free radical scavenging activity.

Total Flavonoid Content was determined by $AlCl_3$ method is presented in Table 1 (the standard curve equation: Y = 0.001x + 0.009 R^2 = 0.994). The content of flavonoids ranged between 10.24-17.4 mg QE/g showing the differential distribution in the extracts. A comparatively higher amount of total flavonoid contents was found in methanolic extract.

Total alkaloid content was determined by the Fazel et al method is presented in (Table 1). The content of alkaloids ranged between 13.44 and 18.2 mg/g dried extract showing the differential distribution in the extracts. A comparatively higher amount of total alkaloid contents was found in methanol, ethyl acetate, and hexane extracts. The hexane extract was found with the least alkaloid content comparatively.

Table 1. Total Phenolic, flavonoid, and alkaloid content

Results are expressed as Mean ± S.D., (n=3)

S. No	Name of the Extracts	TPC(mg GAE/g dried extract)	TFC(mg QE/g dried extract	TAC (mg A/g dried extract)
1	Hexane extract	22.79±0.23	10.24±0.98	13.44±0.42
2	Ethyl acetate extract	55.32±0.10	21.47±0.32	14.86±0.77
3	Methanolic extract	87.58±0.25	17.4±0.42	18.2±0.35

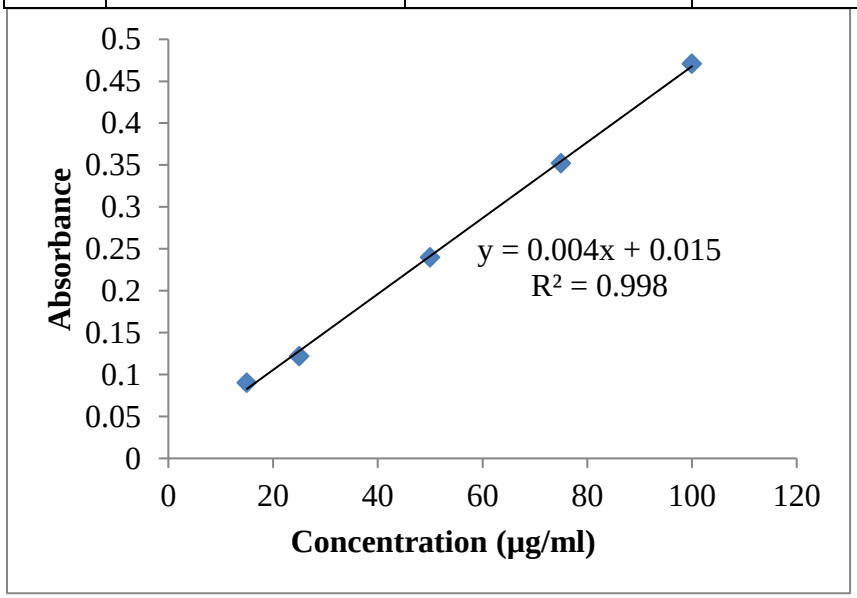

Fig. 1. Calibration curve of Gallic acid

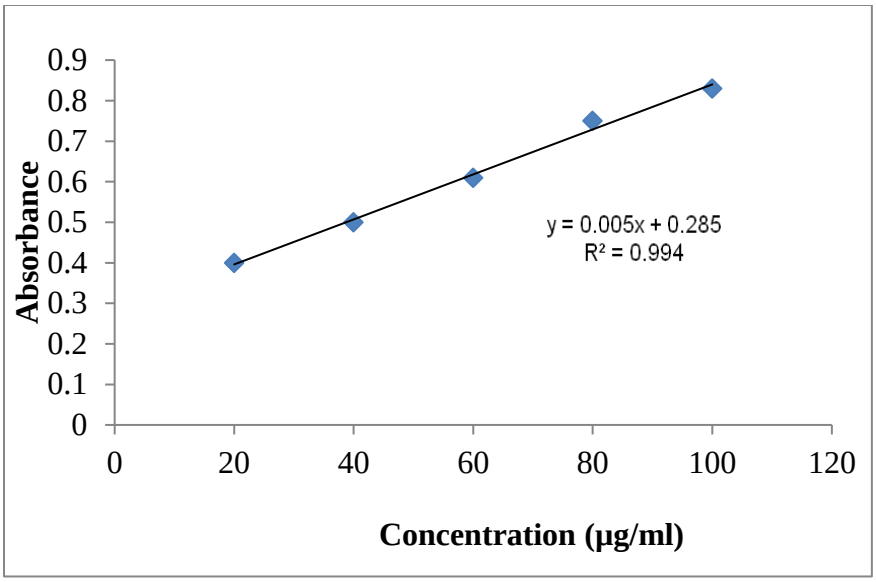

Fig. 2. Calibration curve of Quercetin

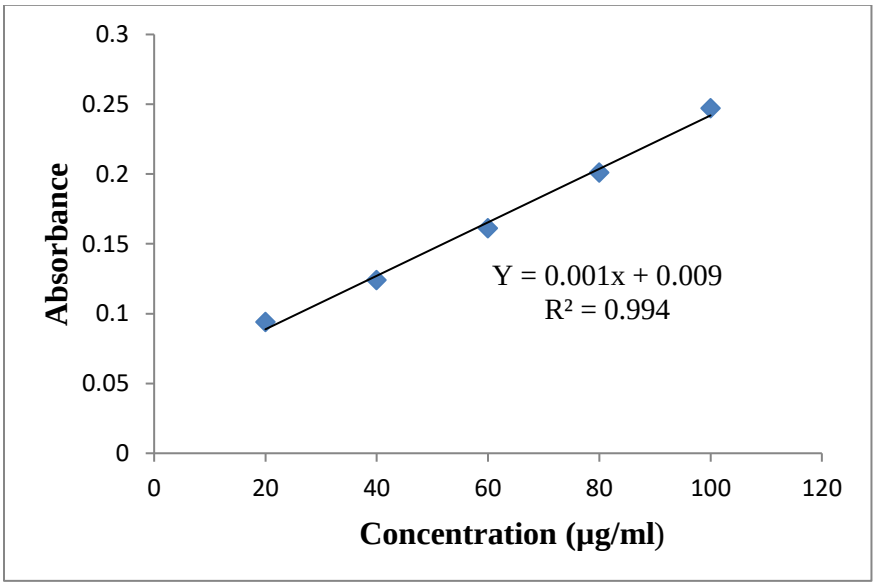

Fig. 3. Calibration curve of Atropin

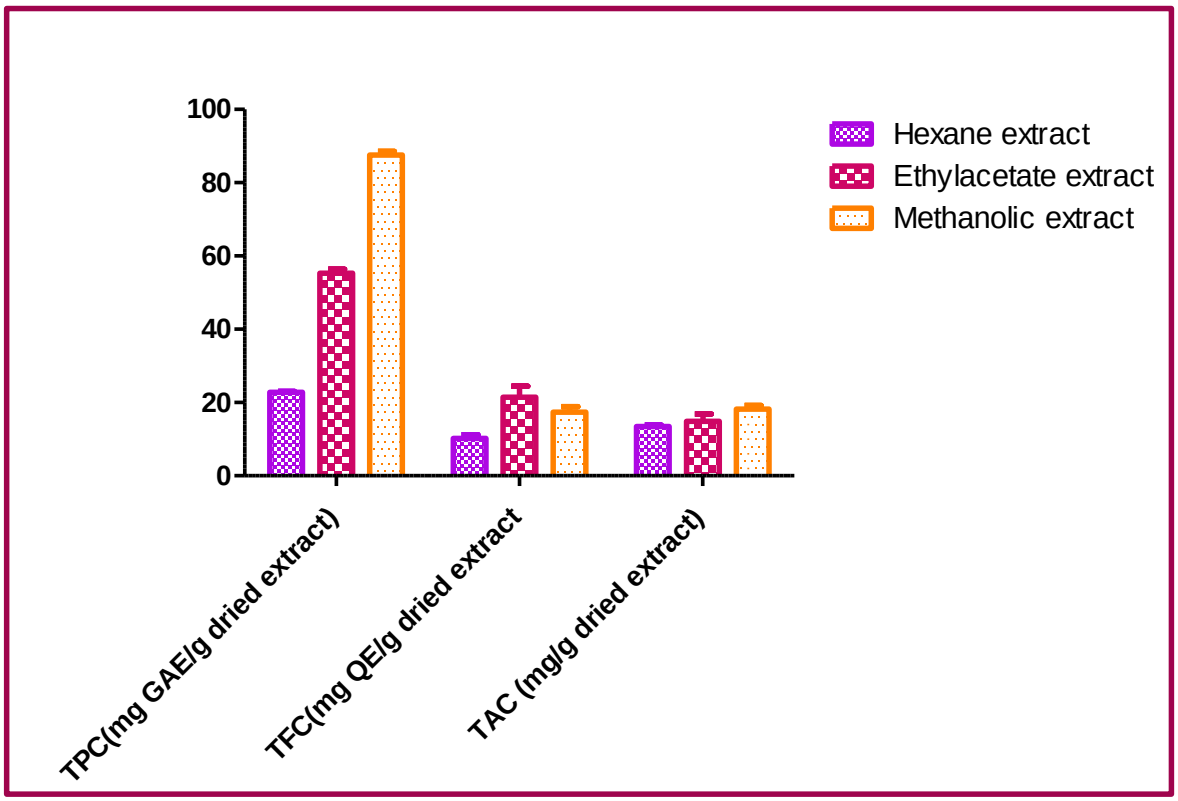

Fig. 4. Total phenolic, flavonoid, and alkaloid content

Conclusion

From the results, it can be concluded that the successively extracted methanolic extract of Grewia tiliaefolia Vahl leaf has a good amount of phenolic, flavonoid, alkaloid content.

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