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SPECTROPHOTOMETRIC QUANTIFICATION OF TOTAL PHENOLIC, FLAVONOID, ALKALOIDES AND GLYCYRRHIZIN CONTENTS IN VARIOUS EXTRACTS OF *ABRUS PRECATORIUS* (L.) AND *CORDIA WALLICHI* (D.) LEAVES

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ABSTRACT

Objective: The aim of the present work was to assess the total phenolic content (TPC), total flavonoid content (TFC), and total tannin content (TTC) of aqueous, hydro-alcoholic and methanolic extracts of *Abrus precatorius*(L.) and *Cordia wallichi*(D.) leaves. Gunja (*Abrus precatorius*) is commonly known as Crabes Eye or Wild Liquorice Root, is a plant species belonging to the family Fabaceae (Papilionaceae). The genus *Cordia*, especially especially *Cordia dichotoma, C. wallichi, C. oblique,*(Boraginaceae) among others, has long been used in traditional medicine as well as used extensively in many Ayurvedic preparations with significant therapeutic significance. **Methods:** The amount of total phenols was estimated using Folin–Ciocalteu assay, the amount of total flavonoids by aluminum chloride assay, total alkaloids by bromocresol green-complex assay and Total glycyrrhizin by reference UV estimation method. **Results:** From the above estimation it was observed that, among all three solvents used for extraction in a series, the contents of total phenolics(TPC), total flavanoides(TFC) total alkaloides(TAC) and glycyrrhizin is highest in the methanolic extract of both the plant leaves. The TPC, TFC and TAC of the methanolic extract of *Abrus precatorius* (L) and *Cordia wallichi* D leaves were5.85±1.4 and 3.65±0.09 mg gallic acid equivalent per g respectively, 1.8 ±0.16 and 2.46±0.22 quercetin equivalent per g respectively, 12±0.16 and 5.8±0.35 atropine equivalent per g, respectively. The glycyrrhizin content in methanolic extract of *Abrus precatorius (*L) was found to be 1.07±0.12 mg of glycyrrhizin equivalent per g of extracts. **Conclusion:** This study gave an insight to the phytoconstituents present in the plant and useful for quantification of the compound in herbal formulations.

KEYWORDS: *Abrus precatorius*, *Cordia wallichi*, Spectrophotometry, total phenolic content, total flavonoid content.

INTRODUCTION

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]

The importance of biological, chemical and pharmacological evaluation of plant-derived bioactive compounds used to cure numerous human ailments has been increasingly recognized in the last few decades, but still there are innumerable potentially useful medicinal plants and herbs waiting to be evaluated and exploited for their effective therapeutic application.[2-5] The medicinal properties of many plants are attributed mainly to the presence of flavonoids, but they may be also influenced by other organic and inorganic compounds such as coumarins, alkaloids, terpenoids, tannins, phenolic acids and antioxidant micronutrients, *e*.g., Cu, Mn, Zn.[6,7]

Based on the strong evidence of biological and pharmacological activities of these bioactive components, the present study was taken up on two alternative medicinal plants namely *A. precatorius* (L.) and *Cordia wallichi* (D.). *A. precatorius* (L.) and *Cordia wallichi* (D.) are woody vine and decodeous trees bearing beautiful seeds and fruits belonging to family Fabaceae and Boraginaceae. Experiments were conducted to determine the total phenolic, flavonoid, alkaloid and glycyrrhizin contents of aqueous, hydro-alcoholic and methanolic extracts of *Abrus precatorius* (L.) and *Cordia wallichi* (D.) leaves.

MATERIALS AND METHODS

Plant Material and Extraction

Chemicals and reagents

All the chemicals and reagents used in the present study were of analytical grade and were obtained from Himedia, Sigma, Merk and S D Fine.

Plant collection and authentication

The authentic plant materials were collected from Kamrup district, Assam and identified and authenticated by Dr.T.G.Gohil, taxonomist and HOD of Botany, Botanist in B.K.M Science College, Valsad (Gujarat). Voucher specimen of the collected plants were prepared and maintained in the Botany department of BKM Science College, Valsad, Gujarat for further reference. Botanical identity was confirmed by correlating their morphological and microscopical characters.

Extract preparation

The leaves of *Abrus precatorius* (L.) and *Cordia wallichi* (D.) were washed repeatedly with distilled water to remove residual material, shade dried, crushed into a coarse powder using an electrical grinder and stored in air tight containers. A weighed amount of the leaf powder was subjected to extraction with solvents such as water, 70% methanol (hydro alcoholic) and methanol in for 7 days, respectively, through cold maceration process. Filtrates obtained from all the solvents were evaporated in rotary evaporator under reduced pressure and were vacuum dried. The dried extracts were packed in airtight containers, labeled and stored in a refrigerator (2-4°C) until needed for the experimental purpose. Aqueous, hydro alcoholic and methanolic extracts of both the plant leaves were used for the present study. After the confirmation of different phytoconstituents by preliminary phytochemical analysis, the extracts were taken for quantitative estimation.

Spectrophotometric measurements

UV/Vis double beam spectrophotometer (Shimadzu UV-1800) and standard quartz cuvetts were used for all the absorbance measurements.

Preparation of standard solution

About 10 mg each of gallic acid, quercetin and glycyrrhizin were accurately weighed into clean and dry volumetric flasks, dissolved in methanol and the volume was made up to 10 ml using the same solvent so as to make the concentration of the solution as 1 mg/ml. Atropine standard solution was taken by dissolving 1 mg pure atropine in 10 ml distilled water.

Preparation of test sample

A stock solution of the test substance was prepared by dissolving 10 mg of dried extracts in 10 ml methanol to give concentration of 1 mg/ml.

Determination of total phenolic content (TPC)[8,9]

Spectrophotometric methods are most commonly used for the quantification of phenolic content. Estimation of total phenol content in the selected plant leaf extract was measured spectrophotometrically by Folin–Ciocalteu colorimetric method, using Gallic acid as the standard and expressing results as gallic acid equivalent (GAE) per g of sample. Preparation of Standard Calibration Curve: 1 ml aliquots of 100 – 500 methanolic Gallic acid solution were mixed with 5 ml of Folin – Ciocalteu reagent (Ten fold diluted) and 4 ml of sodium carbonate (7.5%). The absorbance was read after 30 min at 760 nm.

Estimation of Total Phenolic in extracts: 1 ml of each extract (1000 µg / ml) was mixed with the same reagent as performed above. The absorbance was read after 30 min. at 765 nm for determination of phenolic. All determination was performed in triplicate. Total content (%) of phenolic compound in plant different extracts was calculated as Gallic acid equivalent (GAE): GAE= [(C × V)/M] × 100 where, C=the conc. of Gallic acid established from calibration curve mg/ml; V=Volume of extract (ml); M=the weight of dried plant extracts (mg).

Folin–Ciocalteu is a very sensitive reagent containing phosphomolybdate and phosphotungstate that form blue-complex in alkaline solution by the reduction of phenols. This blue color was measured spectrophotometrically.

Determination of Total flavonoid content[10,11]

Total flavonoid content 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 (5, 10, 15, 20 and 25µg/ml) were prepared in the same manner as described earlier. The absorbance for test and standard solutions were determined against the reagent blank at 359 nm with an UV/Visible spectrophotometer. The total flavonoid content was expressed as mg of QE/g of extract.

The absorbance of each mixture was determined at 359 nm against the same mixture but without leaf extract as a blank. TFC was determined as mg quercetin equivalent per g of sample with the help of calibration curve of quercetin. All determinations were performed in triplicate (n=3).

This method was based on the formation of complex with maximum absorption at 359 nm.

Determination of total alkaloid content (TAC)[12,13]

Preparation of reagents

Bromocresol green solution was prepared by heating 69.8 mg bromocresol green with 3 ml of 2N NaOH and 5 ml distilled water until completely dissolved and the solution was diluted to 1000 ml with distilled water.

Phosphate buffer solution (pH 4.7) was prepared by adjusting the pH of 2M sodium phosphate (71.6 gm Na_2HPO_4 in 1 L distilled water) to 4.7 with 0.2 M citric acid (42.02 gm citric acid in 1 L distilled water).

Atropine standard solution was made by dissolving 1 mg of pure Atropine (AR-grade procured from Sigma Company) in 10 ml distilled water.

Seperation of Alkaloid

The plant extract (1mg) was added 10ml of 2 N HCl and filtered. 1 ml of this solution was transferred to separatory funnel and washed with 10 ml chloroform (3 times). The pH of this solution was adjusted to neutral with 0.1 N NaOH. Then 5 ml of BCG solution and 5 ml of phosphate buffer were added to this solution. The mixture was shaken and complex extracted with 1, 2, 3 and 4 ml chloroform by vigorous shaking, the extract was then collected in a 10 ml. volumetric flask and diluted with chloroform. The total alkaloid content was expressed as mg of AE/g of extract.

Preparation of standard curve

Accurately measured aliquots (0.2, 0.4, 0.6, 0.8and 1ml) of Atropine standard solution was transferred to different separatory funnels. Then 5 ml of pH 4.7 phosphate buffer and 5 ml of BCG solution was taken and the mixture was shaken with extract with 1, 2, 3, and 4 ml of chloroform. The extracts were then collected in 10 ml volumetric flask and then diluted to adjust solution with chloroform. The absorbance of the complex in chloroform was measured at spectrum of 470 nm in UV-Spectrophotometer (SHIMADZU UV-1800) against the blank prepared as above but without Atropine.

Atropine standard solution was made by dissolving 1 mg of pure Atropine (AR-grade procured from Sigma Company) in 10 ml distilled water.

Determination of Glycyrrhizin in Abrus precatorius[14]

Preparation of Standard Stock Solution of Glycyrrhizin: Standard glycyrrhizin (100 mg) was dissolved in 100 ml of methanol to prepare stock solution with concentration of 1000µg/ml.

Preparation of Dilution: For the preparation of calibration curve a series of dilution with concentration of 10, 20, 30, 40, 50,60,70,80 µg/ml were prepared by taking aliquots of 0.1, 0.2, 0.3, 0.4, 0.5.0.6,0.7,0.8 ml of stock solution (1000 µg/ml) and diluted up to 10 ml with methanol in 10 ml volumetric flask. The absorbance of glycyrrhizin standard solutions was measured at 248 nm against methanol as blank. Calibration curve was plotted between absorbance and concentration.

Preparation of Sample Solution:- 1000 µg/ml solution of Methanolic extracts of *Abrus precatorius* was prepared and absorbance were measured at 248 nm.The total glycyrrhizin content in the methanolic extract of *Abrus precatorius* was expressed as milligs of glycyrrhizin equivalent per gm of extract.

Statistical analysis

All the determinations were replicated in three independent assays, and the results were reported as a mean ± standard deviation.

RESULTS AND DISCUSSION

The present study has been carried out for quantification of the total phenolic, flavonoid, alkaloid and glycyrrhizin contents of aqueous, hydro-alcoholic and methanolic extracts of *Abrus precatorius* and *Cordia wallichi* leaves. The content of the phenolic compounds in the crude aqueous,hydro-alcoholic and methanolic extracts of *Abrus precatorius* and *Cordia wallichi* leaves, were determined from regression equation of calibration curve (y=0.010x+0.005, R2=0.998) and expressed in gallic acid equivalent were found to be 2.15±0.17, 3.35±0.57 , 5.85±1.4 and 1.05±0.95, 1.78±0.90 ,3.65±0.09 respectively.

The concentration of flavonoids (mg/g) in quercetin equivalent determined from regression equation of calibration curve (y=0.008x+0.076, R^2=0.997) were found to be0.44±1.40, 0.84±0.57, 1.8±0.16 and 0.92±0.14, 1.42±0.18, 2.46±0.22 respectively, the total amount of alkaloid as determined using the regression equation of calibration curve (y=0.005x+0.092, R^2=0.999) and expressed in atropine equivalent were found to be5±0.47, 6±0.65, 12±0.55 and 2.2±0.02, 2.4±0.16and 5.8±0.85 mg/g of plant extract respectively.

The concentration of glycyrrhizin (mg/g) in glycyrrhizin equivalent determined from regression equation of calibration curve (y=0.008x+0.076, R^2=0.997) were found to 0.522±0.35, 0. 88±0.16,1.07±0.12 respectively in *Abrus precatorius* only.

The standard calibration curves of gallic acid, quercetin, atropine and glycyrrhizin are shown in Figs.1-4 respectively. The results are shown in Table 1, 2, 3 and 4.

Table 1: Total phenolic contents in the plant extracts expressed in terms of gallic acid equivalent (mg of GAE/g of extract).

Extracts	mg of GAE/gm of leaf(Abrus)	% of GAE	mg of GAE/gm of leaf(Cordia)	% of GAE
Aqueous	2.15±0.17	0.21%	1.05±0.95	0.105%
70% hydroalcoholic	3.35±0.57	0.33%	1.78±0.90	0.178%
Methanolic	5.85±1.4	0.58%	3.65±0.09	0.365%

Each value is the average of three analysis ± standard deviation; Where GAE is Gallic acid equivalent respectively.

Table 2: Total Flavanoides contents in the plant extracts expressed in terms of Quercetin equivalent (mg of QE/g of extract).

Extracts	mg of QE/gm of leaf(Abrus)	% of QE	mg of QE/gm of leaf(Cordia)	% of QE
Aqueous	0.44±1.40	0.044%	0.92±0.14	0.092%
70% hydroalcoholic	0.84±0.57	0.084%	1.42±0.18	0.142%
Methanolic	1.8±0.16	0.18%	2.46±0.22	0.246%

Each value is the average of three analysis ± standard deviation; Where QE is Quercetin equivalent respectively.

Table 3: Total Alkaloid contents in the plant extracts expressed in terms of Atropine equivalent (mg of TAC/g of extract).

Extracts	mg of AE/gm of leaf(Abrus)	% of QE	mg of AE/gm of leaf(Cordia)	% of AE
Aqueous	5±0.47	0.5%	2.2±0.02	0.22%
70% hydroalcoholic	6±0.65	0.6%	2.4±0.16	0.24%
Methanolic	12±0.55	1.2%	5.8±0.85	0.58%

Each value is the average of three analysis ± standard deviation; Where AE is Atropine equivalent respectively

Table 4: Total Glycyrrhizin contents in the plant extracts expressed in terms of glycyrrhizin equivalent (mg of GE/g of extract).

Extracts	mg of GE/gm of leaf(Abrus)	% of GE
Aqueous	0.522±0.35	0.052%
70% hydroalcoholic	0.88±0.16	0.088%
Methanolic	1.07±0.12	0.107%

Each value is the average of three analysis ± standard deviation; Where GE is Glycyrrhizin equivalent respectively.

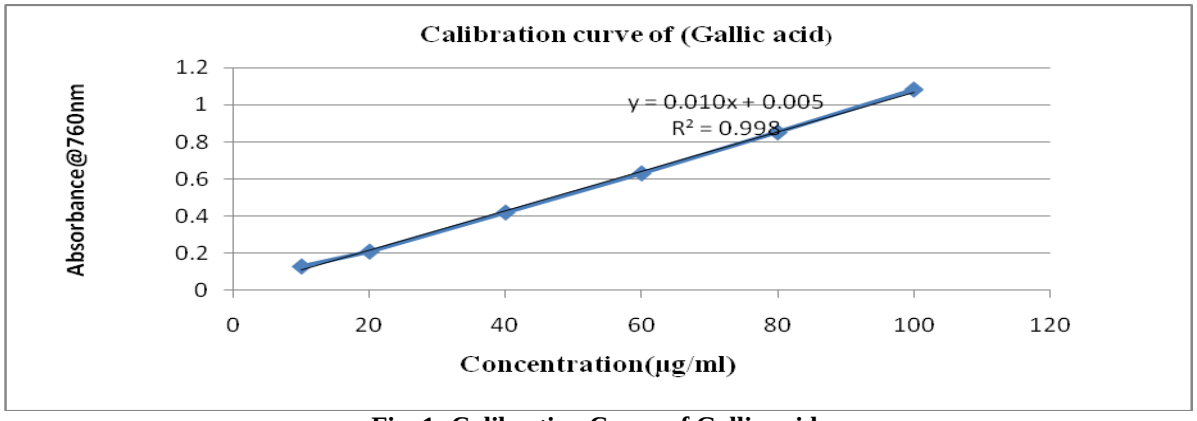

Fig. 1: Calibration Curve of Gallic acid.

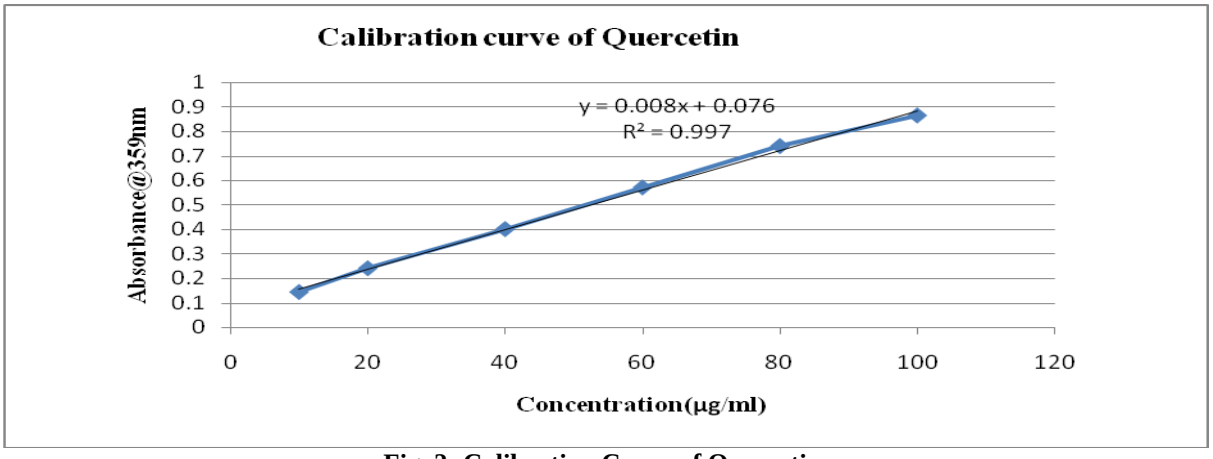

Fig. 2: Calibration Curve of Quercetin.

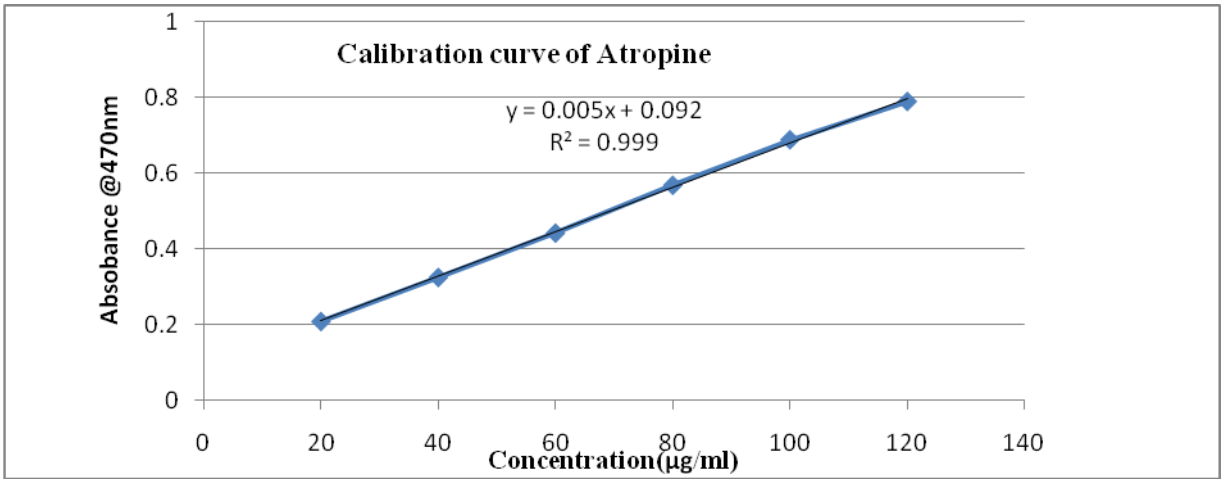

Fig. 3: Calibration Curve of Atropine.

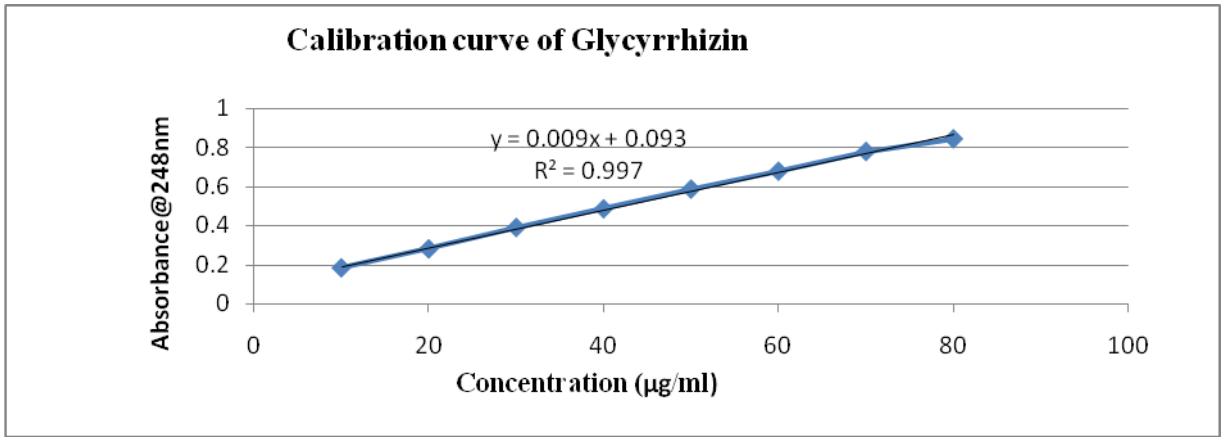

Fig. 4: Calibration Curve of Glycyrrhizin.

CONCLUSION

From the above estimation, it can be concluded that both the plant leaves are rich in phenolic, flavonoid alkaloid compounds and glycyrrhizin is present only in *Abrus precatorius* leaves, and therefore, has provided some biochemical basis for the ethno medicinal use of the sample extracts. As a promising source of bioactive compounds, it can be an excellent source of useful drugs. Moreover, it can also be concluded that the methanolic extracts can also serve as much potent agent than the aqueous and hydro alcoholic extracts due to high contents of bioactive phytoconstituents. .Hence the active methanolic extracts of *Abrus* and *Cordia* were selected for further HPTLC and HPLC estimation and quantification.

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