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Qualitative and quantitative estimation of phyto constituents in different solvent extracts of leaf of *Tabernaemontana divaricata*

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Abstract

Leaves of *Tabernaemontana divaricata* extracts in petroleum ether, ethanol chloroform, methanol and aqueous were analyzed for their phytochemical screening. Qualitative and quantitative phytochemical analysis was performed to identify the presence of various phytoconstituents with standard procedures. The presence of Alkaloids, Glycosides, Flavonoids, Diterpenes, Phenol, Proteins, Carbohydrate, Saponins and Tannins. For quantitative estimation of total phenols, total flavonoids, total alkaloid, total chlorophyll and total ascorbic acid were studied. The present study concluded that *T. divaricata* plant has the ability to cure a variety of diseases because it contains valuable phytochemicals.

Keywords: *Tabernaemontana divaricata*, leaf, phytochemicals, phytochemical screening, qualitative analysis, quantitative analysis

1. Introduction

The *Tabernaemontana divaricata* plant is an evergreen shrub growing to a maximum height of six feet and found in all parts of the India. Normally a Plant produces various metabolic products for their growth and development. Phytochemicals are naturally present in plants and they have biological significances. They play an important role in the plant growth or defending various pathogenic microbes ^[1]. The number of phytochemicals present in plants varies; some produces high amount were some produces less ^[2].

The components which are essential for the growth and survival for the producer plant are known as primary metabolites. Secondary metabolites are plant substances which are derived biosynthetically from primary metabolites. Flavanoids, alkaloids, terpenoids, phenols, tannins, saponins, steroids etc belong to this class. Previous studies have reported the phytochemistry and presence of associated chemicals like alkaloids, terpenoids, steroids, flavonoids, phenyl propanoids, phenolic acids and enzymes in the leaves, stems, and roots ^[3-4].

A variety of chemical compounds extracted from many parts of *Tabernaemontana* species reportedly contain alkaloids, which exhibit biological activities, such as antimicrobial, antioxidant ^[5-6], anti-inflammatory, anticholinesterase, antineurodegenerative, anticancer, antidiabetic, antivenom, larvicidal, antihypertensive, wound healing, analgesic, astringent, antioxidantanxiolytic, anti diabetic and anticonvulsant ^[7-9] and many other activities ^[10-15]. In this study, qualitative and quantitative phytochemical analysis was performed to identify the presence of various phytoconstituents in different leaf extract of *T. divaricata* with standard procedures.

2. Methodology

2.1. Plant material

Leaves of *T. divaricata* were collected from local area of Bhopal in the month of July, 2020. Drying of fresh plant parts was carried out in sun but under the shade and then ground into coarse powder for extraction procedure.

2.2 Extraction Procedure

200 gm of shade dried leaves of *T. divaricata* were extraction with petroleum ether using maceration method. The extraction was continued till the defatting of the material had taken place ^[16-17]. Defatted plant leaf material were extracted in four solvents of different polarity viz water, methanol, ethyl acetate and chloroform by maceration method. The resultant content was filtered with whatman filter paper no.1 and kept for evaporation of solvent to get the dry

concentrated extract. The dried crude concentrated extract was weighed to calculate the extractive yield then transferred to glass vials (6 × 2 cm) and stored in a refrigerator (4°C), till used for analysis [18].

2.3 Determination of Percentage Yield

The percentage yield of yield of each extract was calculated by using formula:

$$\text{Percentage yield} = \frac{\text{Weight of extract}}{\text{Weight of powdered drug taken}} \times 100$$

2.4. Qualitative studies of Phytoconstituents

Phytochemical screening of different solvent leaf extracts was carried out as per the following standard methods [19-21].

2.4.1. Detection of Alkaloids: Extracts dissolved individually in dilute Hydrochloric acid and filtered.

- a) **Hager's Test:** Filtrates were treated with Hager's reagent (saturated picric acid solution). Alkaloids confirmed by the formation of yellow coloured precipitate.
- b) **Wagner's Test:** Filtrates were treated with Wagner's reagent. Alkaloids confirmed by the formation of brown coloured precipitate.

2.4.2. Detection of Carbohydrates: Extracts were dissolved individually in 5 ml distilled water and filtered. The filtrates were used to test for the presence of carbohydrates.

- a) **Fehling's Test:** Filtrates were hydrolysed with dil. HCl, neutralized with alkali and heated with Fehling's A & B solutions. Formation of red precipitate indicates the presence of reducing sugars.

2.4.3. Detection of Glycosides: Extracts were hydrolysed with dil. HCl, and then subjected to test for glycosides.

- a) **Legal's Test:** Extracts were treated with sodium nitropruside in pyridine and sodium hydroxide. Finding of pink to blood red colour indicates the presence of cardiac glycosides.

2.4.4. Detection of Saponins

- a) **Froth Test:** Extracts were diluted with distilled water to 20ml and this was shaken in a graduated cylinder for 15 minutes. Formation of 1 cm layer of foam indicates the incidence of saponins.

2.4.5. Detection of Phenols

- a) **Ferric Chloride Test:** Extracts were treated with 3-4 drops of ferric chloride solution. Formation of bluish black colour indicates the presence of phenols.

2.4.6. Detection of Flavonoids

- a) **Lead acetate Test:** Extracts were treated with few drops of lead acetate solution. Formations of yellow colour precipitate indicate the occurrence of flavonoids.
- b) **Alkaline Reagent Test:** Extracts were treated with few drops of sodium hydroxide solution. Formations of intense yellow colour which become colourless on addition of diluted hydrochloric acid indicates the occurrence of flavonoids.

2.4.7. Detection of Proteins

- a) **Xanthoproteic Test:** The extracts were treated with few drops of conc. Nitric acid. Formation of yellow colour indicates the presence of proteins.

2.4.8. Detection of Tannins

- a) **Gelatin test:** To 1 ml of the plant extract was added few drops of 1% Gelatin solution containing 10% Sodium chloride (NaCl). Formation of white precipitate indicates the presence of Tannins.

2.4.9. Detection of Diterpenes

- a) **Copper Acetate Test:** Extracts were dissolved in water and treated with 3-4 drops of copper acetate solution. Formation of emerald green colour indicate the presence of diterpenes.

2.5. Quantitative Studies of Phytoconstituents

Quantitative studies of phyto constituents were carried out extracts as per the following standard methods [19-22].

2.5.1. Total Phenol Content (TPC) Estimation

The total phenol content of the extract was determined by the modified folin-ciocalteu method. 10 mg Gallic acid was dissolved in 10 ml methanol, various aliquots of 10- 50µg/ml was prepared in methanol. 10 mg of dried extract was dissolved in 10 ml methanol and filter. Two ml (1mg/ml) of this extract was for the estimation of phenol. 2 ml of extract and each standard was mixed with 1 ml of Folin-Ciocalteu reagent (previously diluted with distilled water 1:10 v/v) and 1 ml (7.5g/l) of sodium carbonate. The mixture was vortexed for 15s and allowed to stand for 10 min for colour development. The absorbance was measured at 765 nm using a spectrophotometer.

2.5.2. Total Flavonoids Content (TFC) Estimation

Determination of total flavonoids content was based on aluminium chloride method. 10 mg quercetin was dissolved in 10 ml methanol, and various aliquots of 10-50µg/ml were prepared in methanol. 10 mg of dried extract was dissolved in 10 ml methanol and filter. Three ml (1mg/ml) of this extract was for the estimation of flavonoids. 1 ml of 2% AlCl₃ solution was added to 3 ml of extract or each standard and allowed to stand for 15min at room temperature; absorbance was measured at 420 nm.

2.5.3. Total Alkaloids Content (TAC) Estimation

The plant extracts (1mg) was dissolved in methanol, 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 (40, 60, 80, 100 and 120 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/100mg of extract.

2.5.4. Total Chlorophyll Contents (TCC) Estimation (Aminot, 2000)

One gram of finely cut fresh leaves were taken and ground with 20 – 40ml of 80% acetone. It was then centrifuged at 5000 – 10000rpm for 5mins. The supernatant was transferred and the procedure was repeated till the residue becomes colorless. The absorbance of the solution was red at 645nm and 663nm against the solvent (acetone) blank. The

concentrations of chlorophyll a, chlorophyll b and total chlorophyll were calculated using the following equation:

Total Chlorophyll: $20.2(A_{645}) + 8.02(A_{663})$

Chlorophyll a: $12.7(A_{663}) - 2.69(A_{645})$

Chlorophyll b: $22.9(A_{645}) - 4.68(A_{663})$

2.5.5. Total Ascorbic Acid (TAA) Estimation

Standard calibration curve of ascorbic was established by graphing concentrations versus absorbance of ascorbic standard solutions by taking 10 mL of each of standard solutions (5-25 $\mu\text{g/mL}$) and put in a test tube, then 1 mL of KMnO_4 solution (100 $\mu\text{g/mL}$) was added. This solution was left to stand for 5 minutes. The absorbance of these standard solutions were read at 530 nm against blank. After calibration, Take 10 mL of each of extract sample (1 mg/mL) and put in a test tube, then 1 mL of KMnO_4 solution (100 $\mu\text{g/mL}$) was added. This solution was left to stand for 5 minutes. The absorbances of these standard solutions were read at 530 nm against blank.

3. Results & Discussion

3.1 Qualitative Estimation

The different leaf extracts of *T. divaricata* subjected for preliminary phytochemical screening with solvents chloroform, ethyl acetate, methanol and aqueous, showed a range of secondary metabolites.

The result of the present study made known the presence of Alkaloids, Glycosides, Flavonoids, Diterpenes, Phenol, Proteins, Carbohydrate, Saponins and Tannins in leaf extract. Percentage of % Yield (W/W) of leaf extracts of *T. divaricata* in different five solvents i.e. Petroleum ether, Chloroform, Ethyl acetate, Methanol, Aqueous is depicted in table 1 which shows high percentage of yield (W/W) was found in aqueous extract of leaf. Phytochemical screening of leaf extract of *T. divaricata* in different selected solvent is depicted in table 2.

Alkaloids were detected in methanol and aqueous extract, glycosides were not present in all five solvent extract, flavonoids were present in chloroform, ethyl acetate, methanol and aqueous extracts, Diterpenes were present in all five solvents, phenols were detected in methanol and aqueous extracts, Proteins were present in ethyl acetate, methanol and aqueous extracts, saponins were present in ethyl acetate, methanol and aqueous extracts. Carbohydrate and tannins were not present in all five solvent extracts.

Table 1: % Yield of Leaf extracts of *Tabernaemontana divaricata*

S. No.	Extracts	% Yield (W/W)
1.	Pet. ether	0.268
2.	Chloroform	0.547
3.	Ethyl acetate	0.421
4.	Methanol	3.585
5.	Aqueous	9.580

Table 2: Phytochemical screening of leaf extracts of *Tabernaemontana divaricata*

S. No.	Constituents	Pet. ether extract	Chloroform extract	Ethyl acetate extract	Methanol extract	Aqueous extract
1.	Alkaloids					
	Hager's Test:	-ve	-ve	-ve	+ve	-ve
	Wagner's Test:	-ve	-ve	-ve	+ve	+ve
2.	Glycosides					
	Legal's Test:	-ve	-ve	-ve	-ve	-ve
3.	Flavonoids					
	Alkaline Reagent Test:	-ve	+ve	+ve	+ve	+ve
	Lead acetate Test:	+ve	-ve	-ve	+ve	+ve
4.	Diterpenes					
	Copper acetate Test:	+ve	+ve	+ve	+ve	-ve
5.	Phenol					
	Ferric Chloride Test:	-ve	-ve	-ve	+ve	+ve
6.	Proteins					
	Xanthoproteic Test:	-ve	-ve	+ve	+ve	+ve
7.	Carbohydrate					
	Fehling's Test:	-ve	-ve	-ve	-ve	-ve
8.	Saponins					
	Froth Test:	-ve	-ve	+ve	+ve	+ve
9.	Tannins					
	Gelatin test:	-ve	-ve	-ve	-ve	-ve

Due to existence of phenolic compounds and flavonoids, plant holds antioxidant activity on human fitness. Phenols, flavonoids and tannins are act as antioxidant compounds which play a role as free radical scavengers. Flavonoids are a set of polyphenolic compounds and exploit the inhibition of oxidative and hydrolytic enzymes [23-25]. Tannins also accelerate the remedy for lesions in addition to irritated mucous membranes [26]. Terpenoids, as vitamins, act as regulators of metabolism and play a protective role as antioxidants along with it acquires antimicrobial, antiallergic and antiinflammatory activity [27]. Saponins seize the unique possession of precipitating then coagulating red blood cells

[28]. According to numerous reports, glycosides retain the ability to lower the blood pressure [29].

3.1 Quantitative Estimation

3.1.1 Total Phenolic Content (TPC) Estimation

total phenolic compounds (TPC) was expressed as mg/100mg of gallic acid equivalent of dry extract sample using the equation obtained from the calibration curve: $Y = 0.011X + 0.011$, $R^2 = 0.998$, where X is the gallic acid equivalent (GAE) and Y is the absorbance graph is shown in figure 1.

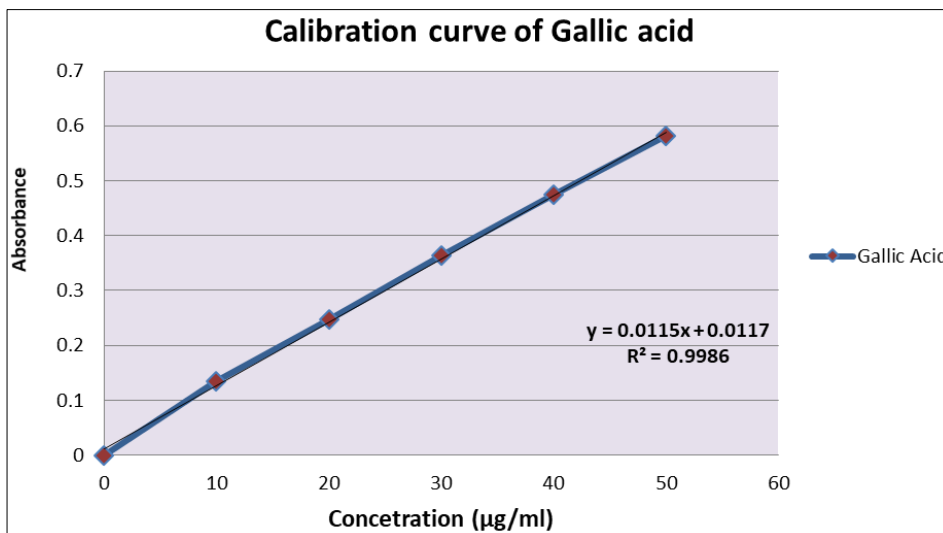


Fig 1: Graph of Calibration curve of Gallic acid for Total Phenolic Content (TPC) Estimation

3.1.2. Total Flavonoids Content (TFC) Estimation

Total flavonoids content was calculated as quercetin equivalent (mg/100mg) using the equation based on the

calibration curve: $Y = 0.010X - 0.006$, $R^2 = 0.998$, where X is the quercetin equivalent (QE) and Y is the absorbance graph is shown in figure 2.

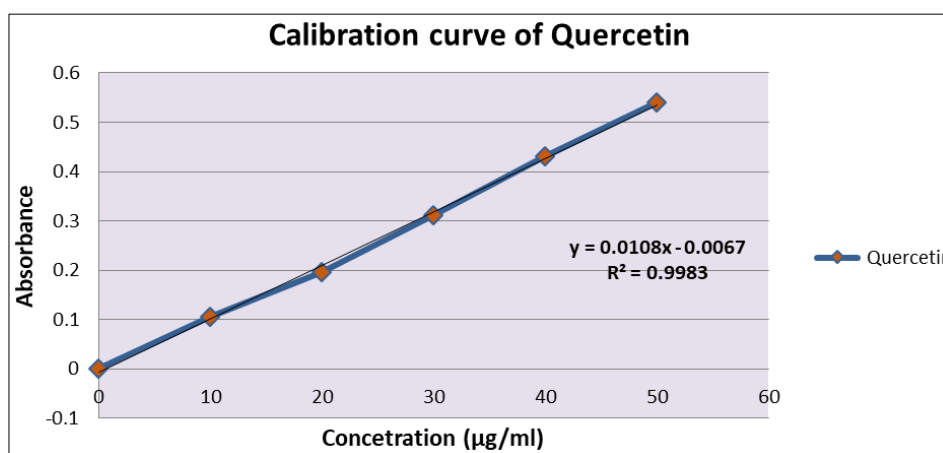


Fig 2: Graph of calibration curve of Quercetin for Total Flavonoids Content (TFC) Estimation

3.1.3. Total Alkaloid Content (TAC) Estimation

Total alkaloid content was calculated as atropine equivalent mg/100mg using the equation based on the calibration curve:

$Y = 0.007X + 0.024$, $R^2 = 0.995$, where X is the Atropine equivalent (AE) and Y is the absorbance graph is shown in figure 3.

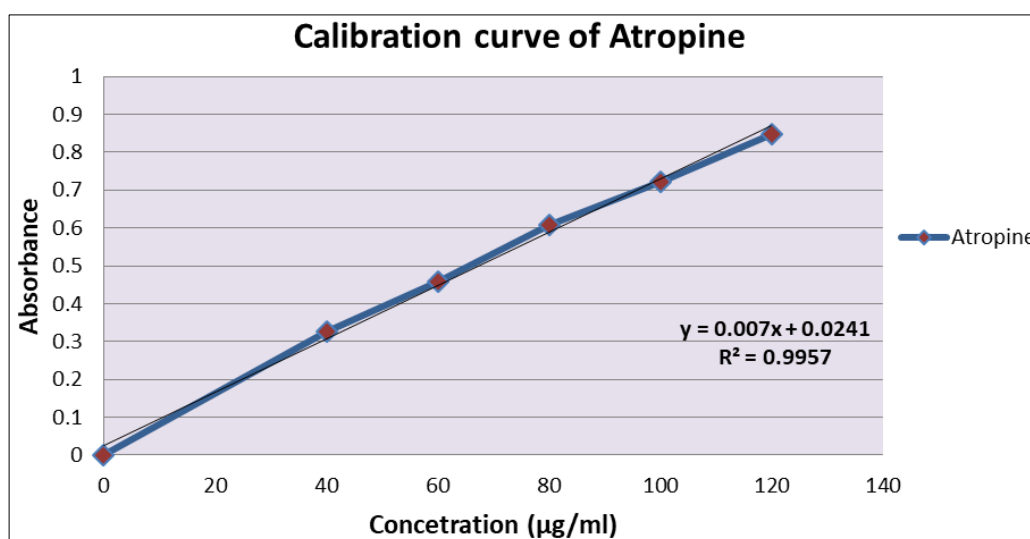


Fig 3: Graph of calibration curve of Atropine for Total Alkaloid Content (TAC) Estimation

Quantitative estimation of phyto constituents in *T. divaricata* leaf extract depicted in table 3.

Table 3: Quantitative estimation of phyto constituents in *T. divaricata* leaf extract

S. No.	Extract	Total Phenolic Content	Total Flavonoids Content	Total Alkaloid Content	Total Ascorbic Acid Content	Total Chloroform Content
(mg/100mg of dried extract)						
1.	Chloroform	-	2.07	-	0.164	3.367 (Chlorophyll a-2.731 and Chlorophyll b-0.636)
2.	Ethyl acetate	-	3.54	-	0.332	
3.	Methanol	0.22	3.47	1.11	0.271	
4.	Aqueous	1.53	3.78	-	0.257	

Total phenolic content 0.22 mg /100mg and 1.53 mg /100mg were estimated in Methanol and Aqueous extract respectively. Total flavonoids content 2.07 mg /100mg in Chloroform extract 3.54 mg /100mg in Ethyl acetate extract, 3.47 mg /100mg in Methanol extract extract, and 3.78 mg /100mg in aqueous extract was estimated. Total alkaloid content 1.11 mg /100mg were estimated in Methanol extract. Total Ascorbic acid content 0.164 mg /100mg in Chloroform extract .332 mg /100 mg in Ethyl acetate extract, 0.271 mg /100mg in Methanol extract extract, and 0.257mg /100mg in aqueous extract was estimated. Total chloroform content 3.367 mg/100 mg was detected in fresh leaf extract.

4. Conclusion

The present study concluded that the presence of phytochemicals in leaves of *T. divaricata* with standard procedures. From the both qualitative and quantitative phytochemical results, it is concluded that leaf extracts of *Tabernaemontana divaricata* has a high quantity of alkaloids, flavinoids and Phenolic compounds, which implies this part of the plant efficiently be used as a potential source for identifying novel phytocompounds which can have a good antioxidant activity and can be used in pharma industries for producing a potent drug candidate. This study highlights some more biologically active constituents which are worthy of further exploration.

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