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QUALITATIVE AND QUANTITATIVE ESTIMATION OF BIOACTIVE COMPOUNDS OF *EUPHORBIA THYMIFOLIA* L.

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Abstract

The present study was aimed to investigate the qualitative and quantitative analysis of the major bioactive constituents of medicinally important plant *Euphorbia thymifolia* in its aqueous extract of whole parts of the plant. Studies were carried out in terms of aqueous extraction, total extractive values, qualitative and quantitative estimation of phytochemicals. The percentage value of yield extraction was found to be 4.56 %. The preliminary phytochemical analysis showed the presence of phenols, flavonoids, saponins, carbohydrates, diterpenes and glycosides. The total phenolic content ranged from 0.998 mg/100mg of dry weight of extract, expressed as gallic acid equivalents. The total flavonoid concentrations varied from 2.12 mg/100mg, expressed as quercetin equivalents. It signifies that results revealed the presence of various bioactive constituents which could be exploited for their potential applications for medicinal purposes.

Keywords: *Euphorbia thymifolia*, Extraction, Phytochemicals, Total Phenolic Content, Total Flavonoid Content.

INTRODUCTION:

The current scenario exhibits the demand for plant drugs throughout the world because of its valuable phytochemicals. The traditional medicine involves the use of extracts of various plants, which are found to have various medicinal properties. Not only the traditional medicine like Ayurveda and Siddha use these plant extracts, but in recent times the allopathic medicine is focusing on using plant extracts to develop medicine which shows more improvement and cures the disease without any side effects. *Euphorbia thymifolia* Linn. is usually referred to as 'laghududhika' or 'chhoti-dudhi'. *Euphorbia thymifolia* belongs to the family Euphorbiaceae, which has around 7500 species in about 300 genera. The plants under Euphorbia genus are used to treat cancer, migraine, warts, intestinal parasites, tumors, etc. The use of *Euphorbia thymifolia* in curing many ailments are increasing as more and more properties of this plant is being found due to advanced research. The *E. thymifolia* is found in tropical regions.[1] This plant is present in the wastelands, along roadsides and wall sides in humid conditions, abandoned fields, etc.[2]

In India, the plant is found in the hills and plains. *Euphorbia thymifolia* is found usually in two ecotypes as green and red forms. These two forms interbreed among themselves and results in forming three intermediates. These are categorized into two major ecological groups like obligate calcifuges and facultative calcicoles. The traditional use of this *Euphorbia thymifolia* is mainly due to its actions

involving laxative, aromatic, sedative, blood purification, anti-viral, antihelminthic, anti-inflammatory, anti-spasmodic, anti-fungal, anti-bacterial, anti-microbial, diuretic properties etc. [3]

Now a day's new technology has made it possible to identify, screen and isolate these active compounds. The chromatographic and spectral fingerprints play an important role in the quality control of complex herbal medicines. Thin layer chromatography (TLC) is the first step to identify the phytochemical compounds present in the sample. With this background an attempt has been made to establish the phytochemical constituents like phenols, flavonoids, glycosides, terpenoids and saponins present in the aqueous extract of *Euphorbia thymifolia* through qualitative, quantitative analysis and TLC techniques. These studies will be helpful for the scientific verification of folklore claim with regard to the utility of this plant.

MATERIALS AND METHODS

Reagents

The reagents and solvents used for the extraction, phytochemical analyses and TLC profiling were analytical grade reagents.

Collection and identification of plant

Euphorbia thymifolia plant material was collected from the village, 'Nitardi', located in Shajapur district, Madhya pradesh, during the month of December, 2016. Further plant material was identified and voucher specimen was submitted in 'Herbarium', Department of Botany, Dr. Hari Singh Gour University, Sagar, M.P. and the registration number allotted to *Euphorbia thymifolia* specimen is Herbarium number P1 (bot/BG/201198). The plant material was dried under shade at room temperature for about 15 days. The dried plant sample was powdered by mechanical grinder and sieved to give particle size 40- 100 mm. The powder was stored in polythene bags at room temperature before extraction.

Preparation of extract

Euphorbia thymifolia dried and powdered plant material (50 g) was extracted with Hot continuous percolation method (Soxhlet extraction). The temperature was maintained at 70°C. The extraction was carried out using water as a solvent. The extract was filtered through a paper filter (Whatman, No.1) and evaporated to dryness under reduced pressure by the rotary evaporator. The obtained crude extract was stored in dark glass bottles for further processing.

Yield of the extract obtained was calculated by formula as mentioned below:

Extractive yield value = Weight of concentrated extract/Weight of plant dried powder × 100

Qualitative phytochemical analysis of plant extract

The different qualitative chemical tests were performed for establishing profile of given extract for its chemical composition. The extract was examined for the presence of various phytoconstituents such as carbohydrate, alkaloids, glycosides, saponins, phenolic compound, tannins and flavonoids. All tests were done as per the procedure given in the standard book. [4]

TLC (Thin Layer Chromatography) profile

For the separation of different phytochemical compounds in the aqueous extract of *Euphorbia thymifolia*, the extract was spotted manually using a capillary tube on precoated silica gel G TLC plates (15X5 cm with 3 mm thickness). The spotted plates were put into a solvent system to detect the suitable mobile phase as per the method of Wagner et al..[5, 6] After the separation of phytochemical constituents, the spraying reagents such as Dragendorff reagent, 10% ethanolic sulphuric acid, 10% sulphuric acid, 5% ferric chloride, Kedde reagent, vanillin phosphoric acid reagent and vanillin sulphuric acid reagent were used to identify the respective compounds. The colour of the spots were noted and Rf values were calculated by using the following formula: Retention time (Rf) = Distance travelled by the solute/Distance travelled by the solvent

Quantitative phytochemical analysis

Total phenols determination

The amount of total phenolic contents of extract of *Euphorbia thymifolia* was determined by the spectrophotometric method of Kim et al., [7] with slight modification. A diluted plant extract (1 ml) or Gallic acid standard phenolic compound was added to a 25 ml volumetric flask, containing 9 ml of distilled water. 1 ml of Folin-Ciocalteu's phenol reagent was added to the mixture and shaken. After 5 min, 10 ml of 7% Na_2CO_3 solution was mixed in to the test sample. The solution was diluted to 25 ml distilled water and mixed thoroughly. The mixture was kept in the dark for 90 min at 23°C, after which the absorbance was read at 750 nm. Total phenol content was determined from extrapolation of calibration curve which was made by preparing Gallic acid solution. The estimation of the phenolic compounds was carried out in triplicate. The Total phenolic content was expressed as milligrams of Gallic acid equivalents (GAE) per gram of dried sample.

Total flavonoids determination

The total flavonoids assay was conducted according to Katasani Damodar.[8] Total flavonoids content was determined by using aluminium chloride colorimetric method. Aqueous extract (0.5 ml) was mixed with 1.5 ml of methanol, 0.1 ml of 10% aluminum chloride, 0.1 ml of 1M potassium acetate and 2.8 ml of distilled water. It remained at room temperature for 30 minutes. The absorbance of the reaction mixture was measured at 510 nm using UV-Visible spectrophotometer. The calibration curve was prepared by

preparing quercetin solutions at concentrations 2 to 8 µg/ ml in methanol. The Total Flavonoids Content was expressed as milligrams of quercetin equivalents per gram of dried sample.

RESULTS AND DISCUSSION

The present study was oriented towards the qualitative and quantitative analysis of the species, *Euphorbia thymifolia* and development of fingerprints using TLC technique.

Determination of percentage yield

The crude extract so obtained after the soxhlet extraction process was further concentrated on water bath evaporation of the solvents completely to obtain the actual yield of extraction. To obtain the percentage yield of extraction is very important phenomenon in phytochemical extraction to evaluate the standard extraction efficiency for a particular plant, different parts of same plant or different solvents used.

Table 1: % Yield of plant material

S. No.	Extract	% Yield (W/W)
1	*Euphorbia thymifolia* L., (aqueous extract)	4.56

Phytochemical screening of extract

A small portion of the extract was subjected to the phytochemical test using Kokate (2004) methods to test for alkaloids, glycosides, tannins, saponins, flavonoids, amino acids and diterpenes separately for plant extract. Small amount of extract was suitably resuspended into the sterile distilled water to make the concentration of 1 mg per ml. The outcomes of the results are discussed separately in the table 2.

Table 2: Results of phytochemical screening

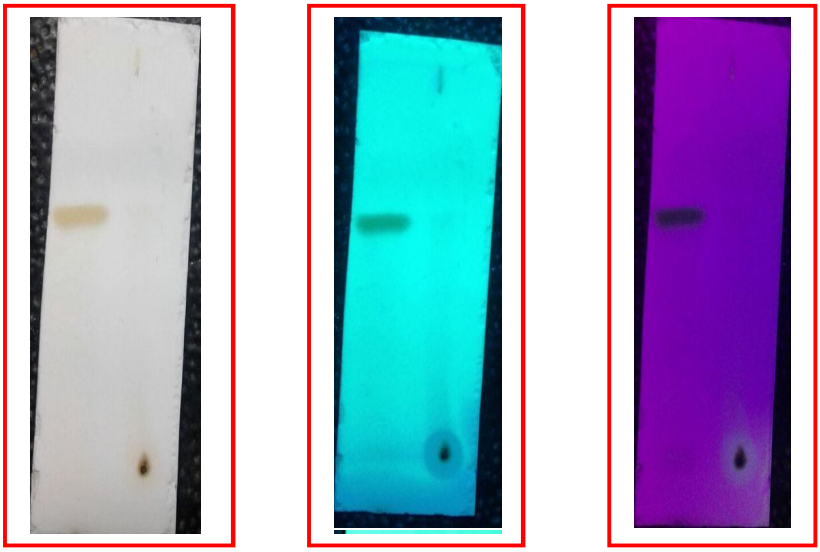
S. No.	Chemical Tests	*Euphorbia thymifolia*	Observation
1.	**Alkaloids**		
	Mayer's reagent	-	Yellow colored precipitate
	Hager's reagent	-	Yellow colored precipitate
	Wagner's reagent	-	Brown/reddish colored precipitate
	Dragendorff's reagent	-	reddish colored precipitate
2.	**Glycosides**		
	Legal's test	+	*Pink to blood red colour*
3.	**Phenols/Tannins**		
	Ferric chloride	+	Bluish black colored
4.	**Flavonoids**		

	Lead acetate test	+	Yellow Colored precipitate
	Alkaline reagent test	-	Colorless
5.	**Saponins**		
	Foam test	+	Layer of foam
6.	**Carbohydrates**		
	Fehling's solution test	+	Red colored
7.	**Amino acids**		
	Xantoprotein Test	-	Yellow colored
8.	**Diterpenes**	+	Emerald green

Results of thin layer chromatography of aqueous extract of *Euphorbia thymifolia*

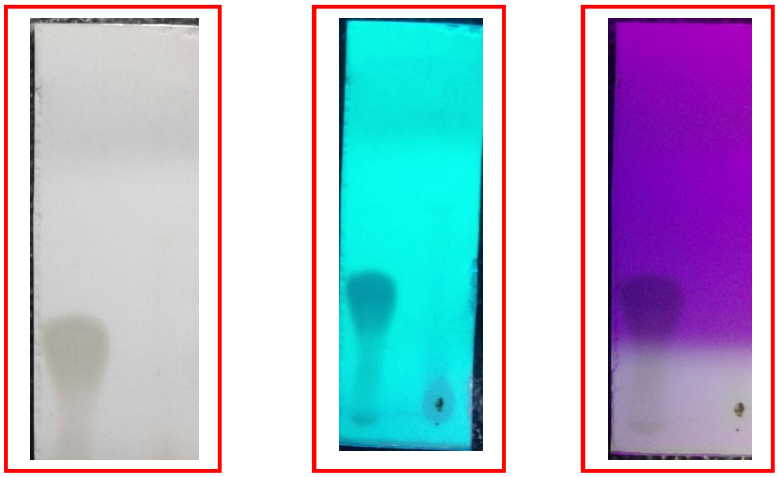
Table 3: Calculation of $R_{f.}$ value

S. No.	Compound	Extract	Rf Value
1.	Gallic acid	Toluene: Ethyl acetate: Formic acid (7:5:1)	0.52
2.	Quercetin	Toluene: Ethyl acetate: Formic acid (5:4:1)	0.76



Normal Light **Short U.V** **Long U.V**

Figure 1: Photograph of T.L.C (Quercetin)



Normal Light **Short U.V** **Long U.V**

Figure 2: Photograph of T.L.C (Gallic acid)

Results of estimation of total phenolic and flavonoids contents

Total phenolic content estimation (TPC)

The content of 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 = 0.002X- 0.016, R^2= 0.981,** where X is the absorbance and Y is the tannic acid equivalent (GAE).

Calibration curve of gallic acid

Table 4: Preparation of calibration curve of gallic acid

S. No.	Concentration	Absorbance
0	0	0
1	25	0.049
2	50	0.093
3	75	0.155
4	100	0.255
5	125	0.315
6	150	0.421

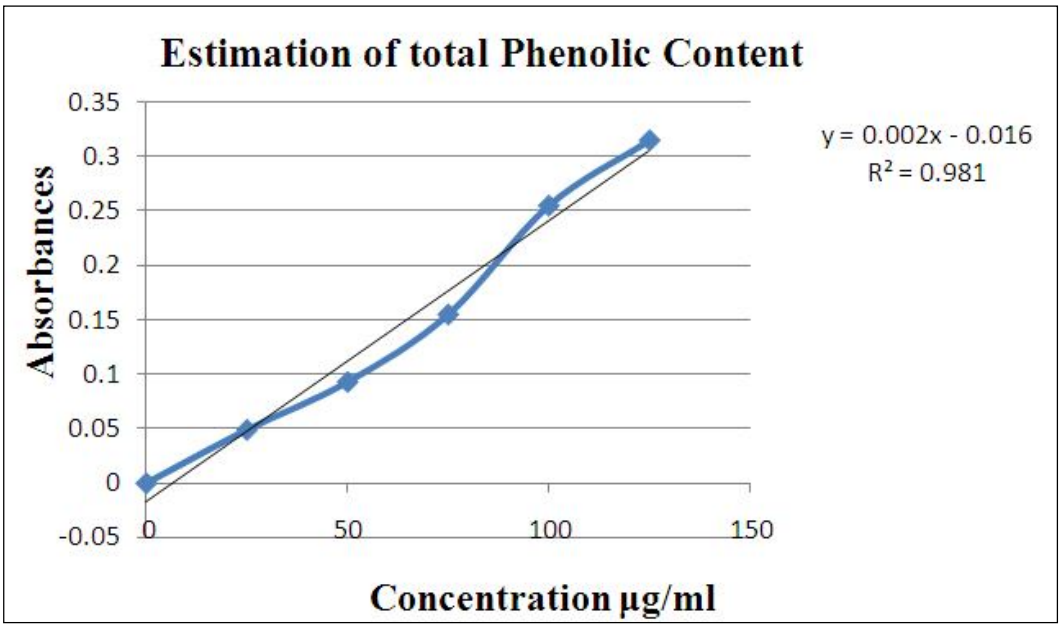


Figure 3: Graph of estimation of total phenolic content

Total flavonoids content estimation (TFC)

Total flavonoids content was calculated as quercetin equivalent (mg/100mg) using the equation based on the calibration curve: **Y=0.004 X + 0.003, R^2=0.995**, where X is the absorbance and Y is the quercetin equivalent (QE).

Table 5: Preparation of calibration curve of quercetin

S. No.	Concentration	Absorbance
0	0	0
1	25	0.119
2	50	0.195
3	75	0.297
4	100	0.387
5	125	0.517
6	150	0.626

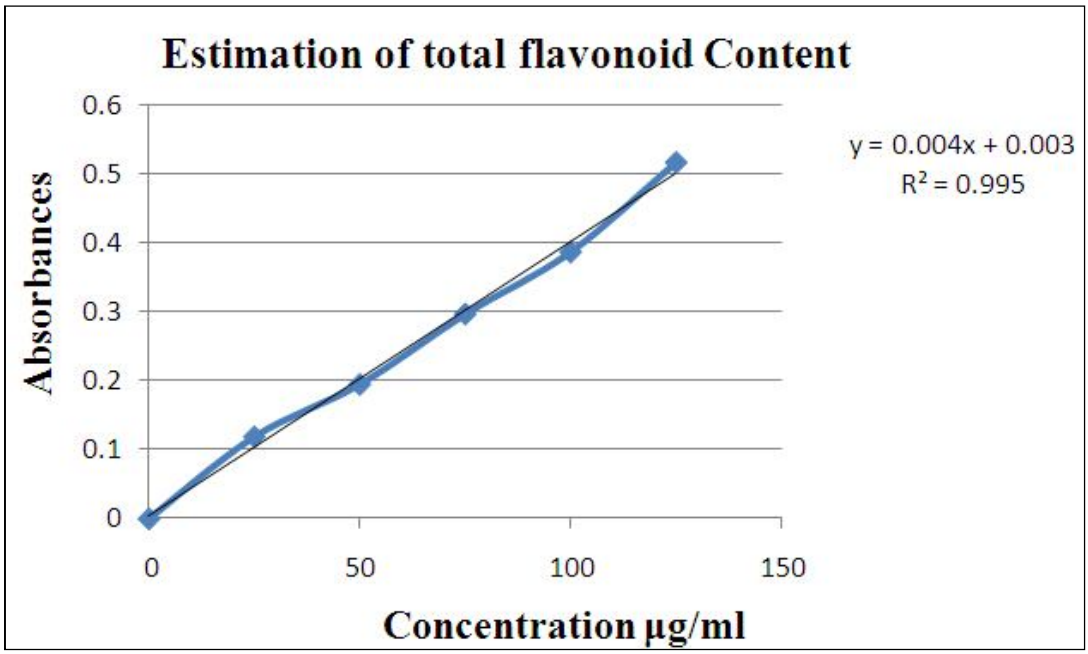


Figure 4: Graph of estimation of total flavonoids content

Results of estimation of total phenolic and flavonoids content

Table 6: Estimation of total phenolics and total flavonoids content

S. No	Extracts	Total phenolic content (mg/100mg of dried extract)	Total flavonoids content (mg/ 100mg of dried extract)
1	*Euphorbia thymifolia* L.	0.998	2.12

Euphorbia thymifolia revealed the presence of phytochemical constituents such as, phenols, flavonoids, saponins, carbohydrates, diterpenes and glycosides but alkaloids and amino acids were absent in the aqueous extracts of *Euphorbia thymifolia* (Table 2). The total phenolic content found in the aqueous extract of *Euphorbia thymifolia* was 0.998 mg/100gm (Table 6). The total flavonoid content was 2.12 mg/100 mg in the aqueous extract, (Table 6). Phenols and flavonoids seemed to have the potential to act as a source of useful drugs and also to improve the health status of the consumers as a result of the presence of various compounds that are vital for good health.[9]

The developed TLC methods will help the manufacturer for quality control and standardization of herbal formulations, such finger printing is useful in differentiating the species from the adulterant and act as biochemical markers for this medicinally important plant species in the Pharma industries and plant systematic studies.[10] The data presented here could be helpful in standardizing extracts of these plants.

Conclusion

This report confirmed the presence of the rich variety of bioactive compounds in the species, *Euphorbia thymifolia* and it could lead for the development of the new pharmaceuticals that address hither to unmet therapeutic needs. For further study, with the help of developing analytical method pure active chemical compound should be isolated and identified on the basis of reference standards.

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