Qualitative Analysis of Primary and Secondary Metabolites in *Euphorbia hirta* L.

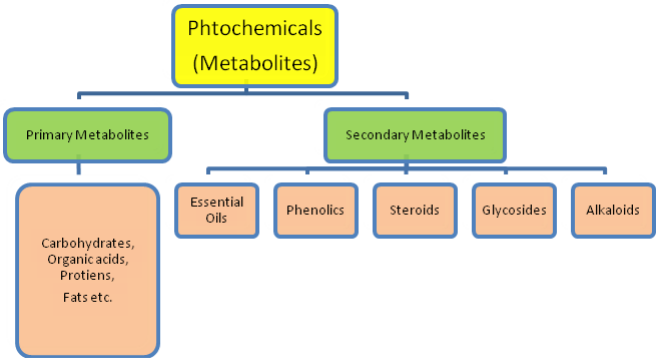
Balchand Baswal[1], O.P. Sharma[2], Rajendra Prasad[3]
[2]Associate Professor
[3]Assistant Professor
[1, 2, 3] Govt. College, Bundi, Rajasthan, India

Abstract- *The chemical compounds produced and accumulated in plants are known as phytochemicals.These phytochemicals protect plants from disorder and damage and are also responsible for other properties like color, taste, flavor, aroma, and odor in plants. Euphorbia hirta L. is an ethnomedicinal plant and widely used in traditional herbal medicine. This paper carried out the qualitative and quantitative analysis of primary and secondary metabolites in Euphorbia hirta L. Various qualitative and quantitative test methods were included for the parameters of primary and secondary metabolites. Euphorbia hirta L. extract showed the presence of all phytochemical constituents.*

Keywords- Benedict's test,Phytochemicals, Soxhlet apparatus

I. INTRODUCTION

Phytochemistry = Phyto + Chemistry (from the Greek word phyto, meaning plant + chemistry meaning study of chemical composition) thus phytochemistry is the study of chemicals produced by plants or biologically active, naturally containing chemical compounds accumulated in plants. These chemical compounds protect plants from disorder and damage and are also responsible for other properties like color, taste, flavor, aroma, and odor in plants. the phytochemicals act as metabolites. Phytochemicals are deposited in different parts of the plants, like in the roots, stems, fruits, seeds, flowers, and leaves. Not only do these phytochemicals control damage and protect plants but also useful for the human to treat various diseases in the body. These properties of phytochemicals are the basis of ethnomedicines. The study of phytochemicals opened a new pathway for the commercial industries like pharmaceutical industries, nutraceutical industries, etc. Polyphenols, flavonoids, isoflavonoids, anthocyanidins, phytoestrogens, terpenoids, carotenoids, limonoids, phytosterols, glucosinolates, and fibers are some important phytochemicals categorized as follows –



Euphorbia hirta L. is a pantropical weed which is also known as asthma weed. Its local name is 'dudhi.' It belongs the family Euphorbiaceae. It is an erect or prostrate, annual, and hairy herb. It grows in open grasslands, and roadsides. Its stem is long, solid and hairy that produces white latex. Leaves of the plant are simple, elliptical, opposite, and hairy with dentate margins. Flowers are unisexual and axillary cymes. Petals are absent in flowers. Fruits of the plant are capsule. Seeds are tiny, oblong, four sided and red color. Root of the plant is white brown taproot. It is used to treat asthma, hypertension, and skin diseases.

II. METHODS AND MATERIALS

Plant Material – The plant material was collected from the village Gudha district Jhalawar, Rajasthan. The whole plant was collected for the analysis of primary and secondary metabolites.

Phytochemical Studies

Phytochemical map out was prepared for the evaluation of the selected plant. Various qualitative methods were included for the parameters of primary and secondary metabolites.

Chemicals Used

All chemicals used in analytical methods and reagents were of analytical grade with the highest purity

available. Following chemicals have been used in phytochemical analysis showing in the table- 01

Copper sulphate	Fuming nitric acid	Ethanol	Phenolphthalein
Ferric chloride	Iodine	α-naphthol (1-naphthol)	Pyridine
Mercuric chloride	Picric acid	Sulfuric acid	Sodium nitroprusside
Sodium hydroxide	Lead acetate	Folin-ciocalteau (f-c) reagent	Potassium hydroxide pellets
Potassium iodide	Hydrochloric acid	Potassium sodium tartrate	Mercury
Sodium carbonate	Acetone	Sodium hydroxide	Chloroform
Catechol	Ammonium hydroxide	Phenol	Potassium sodium tartrate (rochelle salt)

Table 01

Instruments and Apparatus Required

Instruments and apparatus required in different analytical methods are as followsSoxhlet apparatus, Water bath, Test tube shaker, Digital balance (Minimum .01gm), Centrifuge, Hot air oven, Electric mixer grinder, Refrigerator etc.

Preparation of Plant Extract

Freshly collected samples of whole plants were washed thoroughly under tap water and were dried in a hot air oven at 40-50º C for a week. The dried whole plants were pulverized to a fine powder with the help of a sterile electric grinder. The extract was prepared using the Soxhlet apparatus. 60gm of dried powder was extracted for 24 hours in 300 ml ethanol and the process was repeated till colorless solvent was obtained. The colorless solvent indicated the completion of the extraction process. The concise extract was stored and later used for the analysis of primary and secondary metabolites.

Euphorbia hirta L.

Powder of whole plant of *Euphorbia hirta* L.

ANALYSIS OF PRIMARY METABOLITES

Tests for Carbohydrates

Molisch's Test

The test was performed according to the method of Ramakrishnan et al. (1994). A 2 ml of the extract was added with 2 drops of Molisch's reagent. After shaking 2 ml of concentrate sulphuric acid was added along the side of the test tube. At the junction of two solutions, a reddish violet ring evolved which indicated presence of carbohydrates.

Test for Reducing Sugars

Benedict's test

A 1 ml of the extract was mixed with 3 ml of Benedict's solution and heated in a boiling water bath for 10 minutes. After cooling of the solution, a green, yellow, or red precipitate appeared which indicated the presence of reducing sugars.

Tests for Proteins

Millon's Test

The test was performed according to the method of Fisher (1968) and Ruthmann (1970). When a 2 ml of the extract was added with 2 drops of Millon's reagent in a test tube, a white creamy precipitate appeared. On heating the white creamy precipitate, it changed to brick red which indicated the presence of proteins.

Biuret Test

The test was performed according to the method of Gahan (1984). A 2 ml of extract and a few drops of copper sulphate solution were mixed. After this, 1 ml of ethanol (95%) with an excess of potassium hydroxide pellets was added to the solution. A violet or pink layer appeared which indicated the presence of proteins.

Test for Fats and Fixed Oils

Saponification Test

The test was performed according to the method of Kokate (1999). A few drops of extract and 0.5 N alcoholic potassium hydroxide solutions were mixed. After it, a drop of phenolphthalein was added to the solution. Finally, the mixture was heated in a water bath for 2 hours. The formation of soap or partial neutralization of alkali indicated the presence of fixed oils and fats.

ANALYSIS OF SECONDARY METABOLITES

Tests for Alkaloids

50 mg of dried powder of the sample was stirred with a few drops of dilute hydrochloric acid and the solution was filtered. The filtrate was tested carefully using different test methods as follows:

Mayer's Test

The test was performed according to the method of Evans (1997). 4 ml of filtrate was mixed with a few drops of Mayer's reagent by the side of the test tube. A white or creamy precipitate indicated the presence of fixed oils and fats.

Wagner's Test

The test was performed according to the method of Wagner (1993). 4 ml of the filtrate was added with 1 ml of Wagner's reagent. A reddish-brown precipitate confirmed the presence of fixed oils and fats.

Hager's Test

The test was performed according to the method of Wagner et al. (1996). 4 ml of filtrate was added to 1 ml of Hager's reagent. A prominent yellow precipitate appeared which precipitate indicated the presence of fixed oils and fats.

Tests for Tannins and Phenols

Ferric Chloride Test

The test was performed according to the method of Mace (1963). 3 ml of the filtrate was added with 1ml of 5% ferric chloride solution in a test tube. The Bluish black color in the ethanolic layer indicated the presence of tannins and phenols.

Lead Acetate Test

3 ml of the filtrate was added with 1 ml of 10% lead acetate solution. A bulky white precipitate indicated the presence of phenolic compounds.

Test for Flavonoids

Alkaline Test

When 3 ml of the filtrate was added with a few drops of 10% sodium hydroxide solution, the color of the solution changed to yellow. After adding dilute acid, the yellow color disappeared which indicated the presence of flavonoids.
When 3 ml of filtrate was added with a few drops of 10% ammonium hydroxide solution, a yellow fluorescence appeared which confirmed the presence of flavonoids.

Test of Saponins

Froth Test

1ml ethanolic filtrate mixed with, 50 mg sodium carbonate and 1.5 ml distilled water was added to the mixture, shaken vigorously for up to 5 minutes. A honeycomb-like froth formed which was stabled for 15 minutes. It confirmed the presence of saponins.

Test of Terpenoids

Horizon's Test

1 ml of ethanolic filtrate was added to 2 ml of trichloroacetic acid, a yellow to red precipitate appeared which indicated the presence of terpenoids.

Test of Glycosides

Legal Test

1 ml of pyridine and 1ml of sodium nitroprusside were added to 2ml of filtrate. The color of the solution changed to pink or red which indicated the presence of glycosides.

III. RESULTS AND DISCUSSION

Many different tests have been made for qualitative analysis of selected plant species. The results of these tests have been presented under primary and secondary metabolites headings.

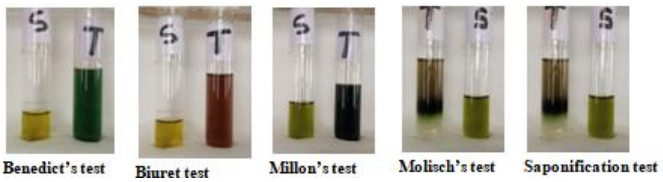
1. RESULTS OF PRIMARY METABOLITES

Table 3 shows the results of different tests for qualitative analysis to find out the presence of primary metabolites (carbohydrates, reducing sugar, proteins and Fats and fixed oils) in the whole plant extract of *Euphorbia hirta* L.

(1) Test results for carbohydrates
- (a) Molisch's test showed the presence of a higher degree of precipitation (+++) in the whole plant extract.
- (b) Benedict's test showed the presence of reducing sugars with a higher degree of precipitation (+++) in the extract.

(2) Test results for protein
- (a) Millon's test showed the presence of a higher degree of precipitation (+++) of protein in the extract.
- (b) Biuret test confirmed the presence of a higher degree of precipitation (+++) of protein in the extract.

(3) Test results for fats and fixed oils
- (a) Saponification test showed the presence of fats and fixed oils with moderate degree of precipitation (++) in the extract.



2. RESULTS OF SECONDARY METABOLITES

(1) Test results for alkaloids
- (a) Hager's test showed the presence of alkaloids with a low degree of precipitation (+) in the extract.
- (b) Mayer's test showed the presence of alkaloids with a low degree of precipitation (+) in the extract.
- (c) Wagner's test confirmed the presence of alkaloids with a low degree of precipitation (+) in the extract.

(2) Test results for phenols and tannins
- (a) Ferric chloride test showed the presence of phenols and tannins with a higher degree of precipitation (+++) in the extract.
- (b) Lead acetate test showed the presence of phenols and tannins with a higher degree of precipitation (+++) in the extract.

(3) Test results for flavonoids
- (a) Alkaline reagent test showed the presence of flavonoids with a moderate degree of precipitation (++) in the extract.

(4) Test results for saponins
- **(a)** Froth test showed the presence of saponins with a moderate degree of precipitation (++) in the extract.

(5) Test results for terpenoids
- (a) Horizon test showed the presence of terpenoids with a moderate degree of precipitation (++) in the extract.

(6) Test results for glycosides
- (a) Legal test showed the presence of glycosides with a higher degree of precipitation (+++) in the extract.

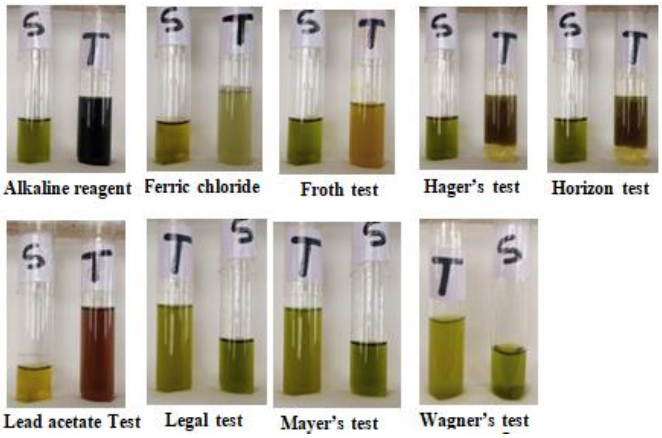


Table-02: Qualitative analysis for primary metabolites in *Euphorbia hirta* L.

S.No.	Name of Phytochemicals	Name of tests	Extracted Part	Results
	Carbohydrates	Molisch's Test	Whole plant	+++
	Reducing sugars	Benedict's Test	Whole plant	+++
	Proteins	Millon's	Whole	+++

		Test	plant	
		Biuret Test	Whole plant	+++
	Fats and Fixed Oils	Saponification Test	Whole plant	++

Table-03: Qualitative analysis for secondary metabolites in *Euphorbia hirta* L.

S.No.	Name of Phytochemicals	Name of tests	Extracted Part	Results
1.	Alkaloids	Mayer's Test	Whole plant	+
		Wagner's Test	Whole plant	+
		Hager's Test	Whole plant	+
2.	Phenols & Tannins	Ferric Chloride Test	Whole plant	+++
		Lead Acetate Test	Whole plant	+++
3.	Flavonoids	Alkaline Reagent Test	Whole plant	++
4.	Saponins	Froth Test	Whole plant	++
5.	Terpenoids	Horizon Test	Whole plant	++
6.	Glycosides	Legal Test	Whole plant	+++

+++ = High degree ++ = Moderate degree
+ = Low degree

The results showed the high presence of primary metabolites like carbohydrates, reducing sugars, proteins, etc. and moderate presence of fats, fixed oils, etc. as well as the high presence of secondary metabolites like phenols, tannins, glycosides, etc. and moderate presence of flavonoids, saponins, terpenoids etc. in ethanol extract. Phytochemical screening of crude extracts of *E. hirta* revealed the presence of bioactive compounds like alkaloids, phenols, saponins, tannins, steroids, flavonoids, and glycosides known for their medicinal and curative properties. Phenolics and flavonoids are responsible for the antioxidant activity of the plant (Jakhar and Dahiya, 2017). Various compounds have been isolated and identified from *E. hirta*, among which, terpenoids, flavonoids, and phenols are the major constituents. These metabolic compounds and crude extracts from *E. hirta* have been screened for pharmacological activities in vivo and in vitro (Huang et al. 2012).

IV. CONCLUSION

The above analytical results showed the high presence of phytochemicals. As these phytochemicals are the basis of ethnomedicines so the higher presence of the phytochemicals mayincrease the medicinal value of *Euphorbia hirta L*. and it may open a new pathway for the commercial industrieslike pharmaceutical industries, nutraceutical industries, etc.

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