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Screening of Phytochemical and *In vitro* activity of *Euphorbia hirta* L.

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ABSTRACT

Phytochemicals are secondary metabolites in one or more parts of the medicinal plants. These have the ability to produce a definite physiological action on the human body. Therefore present study was planned to evaluate TLC and GC-MS analysis of phytochemicals and in vitro antioxidant activity of the leaf extract of Euphoria hirta Linn. The phytochemical constituents were screened through TLC and GC-MS studies and the in vitro antioxidant activity of the alcohol and acetone extract was also investigated. The E.hirta has flavanoids, steroids and phenols which were screened through TLC and GC-MS analysis. In in vitro activity, the highest inhibitory activity of Euphorbia hirta was found in acetone extract when compared to alcoholic extract. The leaves of various extracts of Euphorbia hirta possessed lots of phytochemical constituents and have a potent in vitro antioxidant activity.

Key words: Phytomedicine, *Euphorbia hirta* L., antioxidant study, TLC, GC-MS.

INTRODUCTION

Herbal medicine or phytomedicine refers to the use of any plant seeds, berries, roots, leaves, bark or flowers for medicinal purposes [1]. Plants are used medicinally in different countries and are a source of many potent and powerful drugs [2-5]. Plant drugs could be effective and at the same time have less or no side effect [6]. Now a days 80% people (WHO estimated) from all over world are interested towards traditional medicines [7]. Drug therapy has a profound influence on the health statistics all over the world. Herbal medicine is the most ancient form of health care known to humankind.

The plant *Euphorbia hirta linn* belongs to the family Euphorbiaceae. It is a small annual herb common to tropical countries. It can grow to a height of 40 cm. The plant is commonly called pill bearing spurge and asthma herb [8]. The stem is slender and often reddish in colour, covered with yellowish bristly hairs especially in young parts. The leaves are oppositely arranged, lanceolate and are usually greenish or reddish underneath measuring about 5cm long. The stem and leaves produce white or milky juice when cut [9]. The plant leaves are used to treat colic troubles, dysentery, cough, asthma, worms and vomiting. It has vermifuge properties and it is also eaten as vegetables. The white latex is used as eye drops to cure conjunctivitis. Paste of leaf is applied externally (twice daily) on the place of scorpion bite. *Euphorbia hirta* latex is applied on swellings, piles and boils [10]. Therefore present study was planned to evaluate TLC and GC-MS analysis of phytochemicals and *in vitro* antioxidant activity of the leaf extract of *Euphoria hirta* Linn.

EXPERIMENTAL SECTION

Collection of plant

The leaf of the *Euphorbia hirta linn* were collected from Mannargudi Town, Tamilnadu and shade dried for about three weeks. The plant material was identified by Dr.S. Madhavan, Botanist, S.T.E.T. Women's College, Mannargudi, Tamil Nadu, India. The leaves are then coarsely powdered and were used for the extraction process

Preparation of plant extract

The dried leaves of *Euphorbia hirta* Linn. are moderately coarse powdered and the extraction is carried out by cold maceration process using ethanol, hexane and distilled water for 72 hours. The ethanol, hexane and aqueous extracts are then filtered through muslin cloth and whatman filter paper. The filtrate was concentrated in evaporator at 50°C to obtain residue.

Phytochemical screening study

Ethanol, hexane, methanol and aqueous extracts of *Euphorbia hirta* Linn. were tested to determine the various phytochemical constituents such as alkaloid, flavonoid, steroid, tannin, phenols using TLC and GC-MS (GC 67 – Perkin Elmer) techniques.

Procedure

Thin layer plate preparation

The stationary phase is prepared as slurry with water or buffer at 1:2 and applied to a glass plate or an inert plastic or aluminum sheet, as thin uniform layer by means of a spreader such as glass rod of pipette or using a TLC applicator. (0.25 mm thickness for analytical separations and 2 – 5 mm thickness) for preparative separations are prepared.

Calcium sulphate $CaSO_4$. ½ H_2O; (Gypsum) (10-15%) is incorporated to the absorbent as a binder, as it facilitates the adhesion of the adsorbent to the plate. After application of the adsorbent, the plates are air-dried for 10 – 15 min and then oven – dried for 10 – 15 min at 100°C – 110°C. This process is also known as activation of the adsorbent. The plates can be used immediately or stored in desiccators.

GC-MS

Procedure

Weighed 5g of sample and add 100ml of methanol soaking overnight keep in shaker for one hour. Filter the sample with whatman filter paper No. 1. Concentrate the extract to 1ml at 40°C. The concentrated extract was diluted to 5ml and then inject 1ml of the extract in GC-MS.

The alcoholic extract was subjected to GC-MS analysis. GC analysis was performed on GC clarus 500 perkin Elmer system under the following conditions: Injector temperature 280°C, using a capillary column, Elite 1 (100% Dimethylpoly siloxcane). Oven temperature was programmed from 80 – 280°C at 5°C / min with initial hold of 2 min. The detector was mass detector turbo mass Gold-Perkin Elmer, software – Turbo mass.

1µL alcoholic extract was injected into GC-MS which split the component 10:1. The spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST and MS libraries and ascertain the name, molecular weight and structure of the components of the test materials.

***In vitro* antioxidant study**

Total antioxidant capacity was measured according to spectrophotometric method. 0.1 ml of the extract (10mg/ml) dissolved in water was combined in an test tube with 1ml of reagent solution (0.6M sulfuric acid, 28mM sodium phosphate & 4mM ammonium molybdate). The tubes were capped and incubated in a water bath at 95°C for 90 minutes. After cooling to room temperature, the absorbance of the aqueous solution of each was measured at 695 nm against a blank. Ethanol was used as the standard and the total antioxidant capacity is expressed as equivalent of ascorbic acid.

RESULTS AND DISCUSSION

Phytochemicals are secondary metabolites in one or more parts of the medicinal plants. These have the ability to produce a definite physiological action on the human body. In the present study, leaves of *Euphorbia hirta* were analysed qualitatively. Secondary metabolites such as alkaloids, flavonoids, tannins, steroids, glycosides and carbohydrates were present in *Euphorbia hirta* Linn. But protein, fats and saponins are absent in methanolic extract of *Euphorbia hirta*.

Alcohol, acetone, ethylacetate, hexane, petroleum ether, chloroform, aqueous extracts of *Euphorbia hirta* through Thinlayer chromatography. From this analysis four compounds such as steroid, flavonoid, alkaloid and phenols were found this leaves. The leaves of *Euphorbia hirta* have a high flavonoid and phenolic compounds than the steroid and alkaloid compounds. Hexane and petroleum ether extract contain steroids which was found from its bluish green spot on TLC plate.

Alkaloids are major chemical compound present in leaves of *Euphorbia hirta* as fluorescent blue on TLC plate. Alkaloids are important defense of the plant against pathogenic organisms and herbivores. It also toxin for insects which further modify the alkaloids and incorporate them into their own defense secretion [11].

Flavonoids were found from its greenish yellow spot on TLC plates of Alcohol, acetone and Ethylacetate extract. Phenolic compounds were screened through its orange red spots on TLC plates of alcohol, acetone and aqueous extracts. Generally phenolic pigments are visibly coloured and they are thus particularly easily monitor during their isolation and purification.

GC conditions
Oven program
80°C @ 10°C/min to 150°C (3 min) @ 10°C/min to 280°C (5 min)
Injector temperature : 280°C
Carrier gas : He @ 1ml / min

MS conditions
Mass Type : Full scan mode
Mass range : 40 to 450 Daltons

Figure – 1 *Euphorbia hirta* – Methanol Extract – Chromatogram

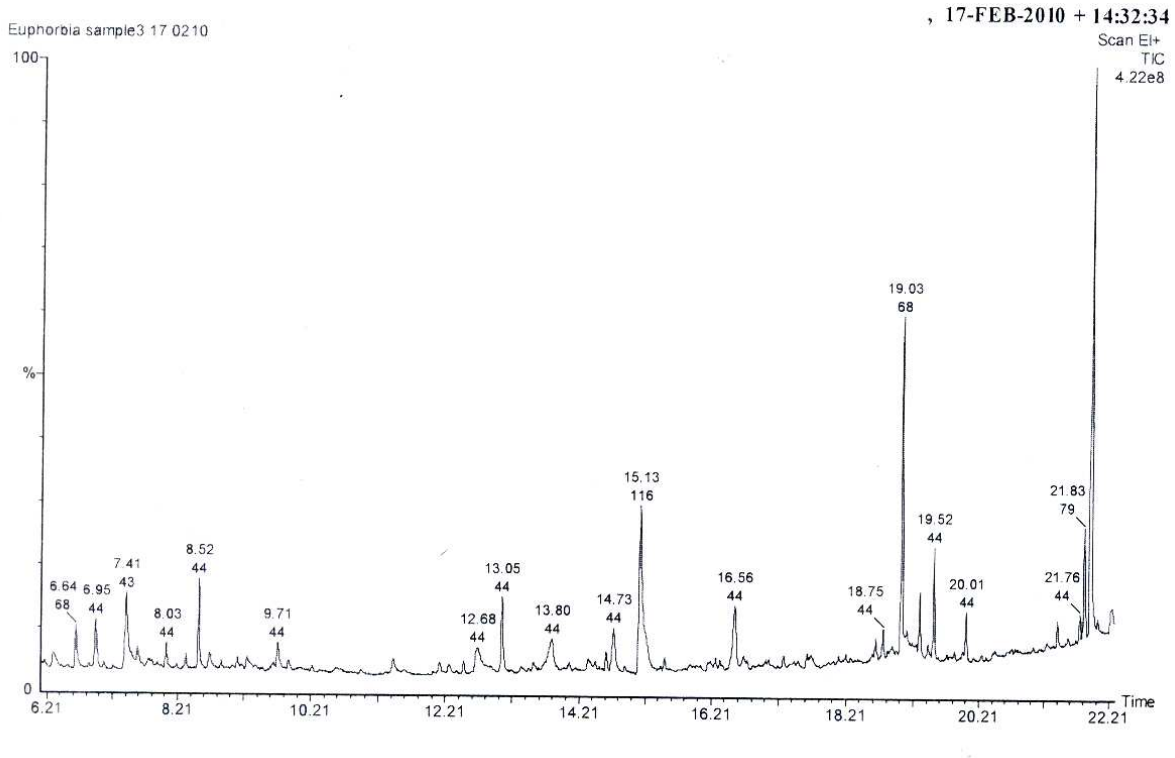

Table – 1

Extract	% of inhibition					
Concentration	**10**	**20**	**40**	**60**	**80**	**100**
Acetone extract	8.23	16.10	27.31	51.68	67.80	94.75
Alcohol extract	10.11	10.86	40.82	58.16	62.54	68.91

The methanolic leaf extract of *Euphorbia hirta* was studied by GC-MS. There are 15 compounds were identified using this technique. Among these, pytol is a major compound

because of its percentage (32%) is high. It is act as a skin care agent and anticancer agent [12]. Hence *Euphorbia hirta* may be used in various diseases such as wounds, boils and skin diseases.

Phytol was a diterpene and their concentration was found to be 32.21%. Phytol is a chemical compound isolated from the plant. It is a liquid alcohol used to synthesize the vitamin E and vitamin K. Phytol is a active ingredient in soaps and cosmetic products. The same report was given by Ciccio [13] and Miyazawa [14]. Terpenoids such as phytol usually have the insecticidal and antihelmintic or antiseptic activity. Hence *Euphorbia hirta* may be used as antiseptic drug.

Further, this study focussed on *in vitro* antioxidant activity of leaf of *Euphorbia hirta* which was achieved by using two different extracts such as alcohol and acetone. Total antioxidant activity of *Euphorbia hirta* is higher in acetone extract compared to alcoholic extract showed in Table – 1.

In conclusion, these studies suggest that the leaves of various extracts of *Euphorbia hirta* possessed lots of phytochemical constituents and have a potent *in vitro* antioxidant activity. Future research is needed to design this herb as a drug after completing the molecular level research work.

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