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Original Article

Phytochemical investigation and standardization of aerial parts of *Euphorbia Helioscopia* L.

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ABSTRACT

The present study has been designed to explore physicochemical properties and standardization of aerial parts of *Euphorbia helioscopia* L. by HPLC method. The various parts of plant have been reported in the treatment of number of pathological conditions such as anti-asthmatic, anti-oxidant, anti-tumor, etc. The active phytoconstituents such as flavonoids, alkaloids, phenolic compound, etc are majorly responsible for these activities. Physicochemical investigation was done by calculating extractive values (water and alcohol soluble), ash values (total ash, acid insoluble ash and water soluble ash), loss on drying and heavy metal content (arsenic, cadmium, lead and mercury). Further, extraction was done by percolation using petroleum ether and methanol successively. HPLC method for standardization of methanolic extract was developed to estimate % of markers present in extract by comparing with standard compounds. Results of present study confirmed percentage of these markers as chlorogenic acid 0.0001%, quercetin 0.00007%, quercetrin 0.081%, rutin 0.010% and luteolin glycoside 0.013%. Hence, it may be concluded that plant extract contains numbers of flavonoids, which further responsible for the various pharmacological activities.

Key words: *Euphorbia helioscopia*, Standardization, flavonoids.

INTRODUCTION

Herbal medicines are the oldest remedies useful for the treatment of various diseases. [1]. Herbal medicine is the mainstay of about 75–80% of the world population, mainly in the developing countries, for primary health care because of better compatibility with human body and lesser side effects [2]. Standardization of herbal medicines is the process of prescribing a set of standards or inherent characteristics, constant parameters, definitive qualitative and quantitative values that carry assurance of quality, efficacy, safety and reproducibility [3]. *Euphorbia helioscopia* belongs to family: Euphorbiaceae, named sun spurge, is a common native plant known since ancient times widely distributed in every continent. The plant has been commonly used as febrifuge, vermifuge, purgative, anthelmintic. Its juice has been commonly applied to warts to cure intolerable pain and associated with sore eyelid [4]. *Euphorbia helioscopia* has been reported to have many pharmacological properties, such as antitussive [5], antitumor [6], anti-allergic, anti-asthmatic [7], antibacterial activity [8], antioxidant activity [9], antifungal activity [10], antiviral activity [11] and antitumor activity [12].

The main objective of the present study was to investigate physicochemical parameters, extraction and standardization of methanolic extract of aerial parts of *Euphorbia helioscopia.*

MATERIALS AND METHODS

Plant Materials: The aerial parts of *Euphorbia helioscopia* was collected from I.I.I.M., Jammu (J&K) in the month of September. The plant was identified and authenticated by botanist, Dr. S.N. Sharma, Department of Taxonomy, I.I.I.M., Jammu, (J&K). After authentication, aerial part was dried at room temperature until they were free from the moisture and subjected to physical evaluation with different parameters.

Determination of physicochemical parameters: Physicochemical values such as the Extractive values, Ash values, percentage of loss on drying

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and Heavy Metals Content were carried out as per the standard method described in WHO (1998).

Extractive values

Determination of water soluble extractive value: Accurately weighed 4 g of coarsely powdered air dried material was taken, in a glass stoppered conical flask. The material was macerated with 100 ml of water specified for the plant material concerned for 6 hours, shaking frequently, and then it was allowed to stand for 18 hours. The contents were filtered rapidly. 25 ml of the filtrate was transferred to the tarred bottom dish and was evaporated to dryness on water bath. Then it was dried at 105^0 C for 6 hours, cooled in a desiccator for 30 minutes and was weighed. The content of extractable matter was calculated in mg/g of air dried material.

Determination of alcohol soluble extractive value: Accurately weighed 4 g of coarsely powdered air dried material was taken in a glass stoppered conical flask. The material was macerated with 100 ml of methanol (100%) specified for the plant material concerned for 6 hours, shaking frequently, and then it was allowed to stand for 18 hours. The content was filtered rapidly. 25 ml of the filtrate was transferred to the tarred bottom dish and evaporate to dryness on water bath. Then it was dried at 105^0 C for 6 hours, cool in a desiccator for 30 minutes and was weighed. The content of extractable matter was calculated in mg/g of air dried material.

Loss on drying (LOD): About 2 g of powder was accurately weighed in a petri-dish and the dish was kept in a hot air oven maintained at a temperature 110^0C for 4 hours until a constant stable weight was recorded. The procedure was repeated. The dish was cooled in a desiccator at room temperature and weighed.

Ash values: Powdered plant materials were subjected to total ash, acid insoluble ash and water soluble ash.

Total ash: Accurately weighed 2 g of dried material was taken in a previously ignited and tared silica crucible. The material was spreaded in an even layer and ignited by gradually increasing the heat to 500-600^0C until it was white, indicating the absence of carbon. Then it was cool in a desiccator and weighed. The content of total ash was calculated in mg/g of air dried material.

Acid insoluble ash: To the crucible containing total ash, 25 ml of hydrochloric acid was added. Crucible was covered with a watch glass and boiled gently for 5 minutes. The watch glass was rinsed with 5 ml of hot water and this liquid was added to the crucible. The insoluble matter was collected on an ash less filter paper and was washed with hot water until the filtrate was neutral. The filter paper containing the insoluble matter was transferred to the original crucible, dried on hot plate and ignited to constant weight. The residue was allowed to cool in suitable desiccator for 30 minutes, and was weighed. The content of acid-insoluble ash was calculated in mg/g of air dried material.

Water soluble ash: To the crucible containing the total ash, 25 ml of distilled water was added and boiled for 5 minutes. The insoluble matter was collected in a sintered glass crucible or on an ash less filter-paper. It was washed with hot water and ignited in a crucible for 15 minutes at a temperature not exceeding 450^0 C. The weight of this residue in mg was subtracted from the weight of total ash. The content of water soluble ash was calculated in mg/g of dried material [13].

Determination of heavy metals content: Weighed quantity of the crushed and powder portion from aerial part of plant was taken in the china dish and heated in an oven at 110ºC to evolve moisture. Then the dried sample was heated in a furnace for 4 hour at 550ºC. The contents of china dish was cooled in desiccators and 2.5 ml 6M HNO_3 was added to the dish to dissolve its contents. The solution was filtered and transferred to a 20 ml flask and diluted to the mark. This sample was used for analysis of heavy metals by flame atomic absorption spectroscopy and final concentrations of heavy metals were determined [14].

Extractions

Methanolic extract: Dried Plant was ground to the coarse powder. The coarsely powdered aerial parts (500 g) were packed in a percolator and first soaked in petroleum ether (5 L) and kept overnight. The extract was drained, filtered and concentrated under reduced pressure using rotary film evaporator. The extraction process was repeated three times more under similar conditions. The combined extract was finally dried in vacuum desiccator and weighed. Successive extraction was carried with drug packed in a percolator, soaked in methanol (5 L) and kept overnight. The extract was siphoned out and concentrated under reduced pressure using rotary film evaporator. The extraction process was repeated three times more under similar conditions. The combined extract was finally dried in vacuum desiccator and weighed.

Phytochemical screening: The methanolic extract of the plant was screened for the presence of various phytoconstituents such as saponin, glycosides, protein, amino acids, alkaloids,

terpenoids, tannins, flavonoids and phenolic compounds [15, 16].

Development of protocol of HPLC method for standardization of methanolic extract

Preparation of extract solution

Sample: *Euphorbia helioscopia* (Aerial) MeOH Ext.: 30.0 mg/3mL

A known weight of extract of sample was dissolved in 3 ml of methanol HPLC grade, centrifuged and filtered through 0.45µm millipore filter.20 µL of the extract solution injected in the HPLC system.

Preparation of standards

Rutin: 1.0 mg/5ml methanol HPLC grade from which10, 20, 30, 40, 50 µL injected in HPLC system for making standard curve.

Quercetin: 4.4 mg/5ml methanol HPLC grade from which 10, 20, 30, 40, 50 µL injected in HPLC system for making standard curve.

Quercetrin: 1.0 mg/5ml methanol HPLC grade from which 10, 20, 30, 40, 50 µL injected in HPLC system for making standard curve.

Luteolin glycoside: 1.0 mg/5ml methanol HPLC grade from which 10, 20, 30, 40, 50 µL injected in HPLC system for making standard curve.

Chlorogenic acid: 1.5 mg/5ml methanol HPLC grade from which 10, 20, 30, 40, 50 µL injected in HPLC system for making standard curve.

Analysis

The samples were analyzed at 30˚C on a Merck RP-18 column (5 µm, 250×4.00 mm ID) by UV detection at 340 nm. The mobile phase consisted of 0.05% TFA in Acetonitrile; 0.05% TFA in acetonitrile (gradient) was delivered at a flow rate of 1 ml/min.

RESULTS

In the present study aerial parts of *Euphorbia helioscopia* have been subjected to physicochemical investigation. The results of different parameters for physicochemical study are shown in the Table 1.

Extraction of dried powdered aerial parts of the plant was done by percolation with petroleum ether and methanol successively. Extractive value of petroleum ether extract and methanolic extract were found to be 3.58% and 7.65% respectively. Protocol of HPLC methods for standardization of methanolic extract was developed to estimate the percentage of markers like Rutin, Quercetin, Quercetrin, Luteolin glycoside and Chlorogenic acid present in extract by preparing standard of these markers as shown in Figure 1. Results of HPLC chromatogram and percentage of markers present in this extract are shown in the Table 2 & 3 respectively.

DISCUSSION AND CONCLUSION

The present study established the standardization of aerial parts of *Euphorbia helioscopia.* The plant has been used in traditional medicine in China for the treatment of malaria, bacillary dysentery and osteomyelitis [17]. Plant has been reported to have various pharmacological activities, such as antitussive**,** antitumor**,** anti-allergic and anti-asthmatic. Literature studies revealed that plant have many therapeutics potencies but no work has been carried out on the standardization by using HPLC protocol.

Physicochemical investigation of plant material was done by calculating various parameters which include extractive values i.e. water soluble (16.70%) and alcohol soluble (21.90%), ash values i.e. total ash (7.6778%), acid insoluble ash (1.5929%) and water soluble ash (3.6892%), loss on drying (7.65%), heavy metals content like arsenic (0.17 mg/kg), cadmium (0.06 mg/kg), lead (2.57 mg/kg) and mercury (BDL of 0.001 mg/kg). Petroleum ether and methanol extract of dried powdered aerial parts of the plant were prepared successively with extractive values of 3.58% and 7.65% respectively. Protocol of HPLC method for standardization of methanolic extract was developed to estimate percentage of markers like Rutin, Quercetin, Quercetrin, Luteolin glycoside and Chlorogenic acid present in extract by preparing standard of these markers.

Therefore, it is concluded from the above results that the *Euphorbia helioscopia* contains high amount of flavonoids content, which are accountable for the antioxidant and anti tumor like actions. The plant may be further explored for its phytochemical screening and isolation of compounds responsible for the huge number of pharmacological activities.

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Conflict of interest: There is no conflict of interest to disclose.

Table 1: Physicochemical investigation of aerial parts of *Euphorbia helioscopia* L.

S. No.	Parameters	Observations
I.	**Extractive Value**	
	Water soluble	16.70%
	Alcohol soluble	21.90%
II.	**Loss on Drying**	7.65%
III.	**Ash values**	
	Total ash	7.6778%
	Acid insoluble ash	1.5929%
	Water soluble ash	3.6892%
IV.	**Heavy Metals**	
	Arsenic	0.17mg/kg
	Cadmium	0.06mg/kg
	Lead	2.57mg/kg
	Mercury	BDL of 0.001mg/kg

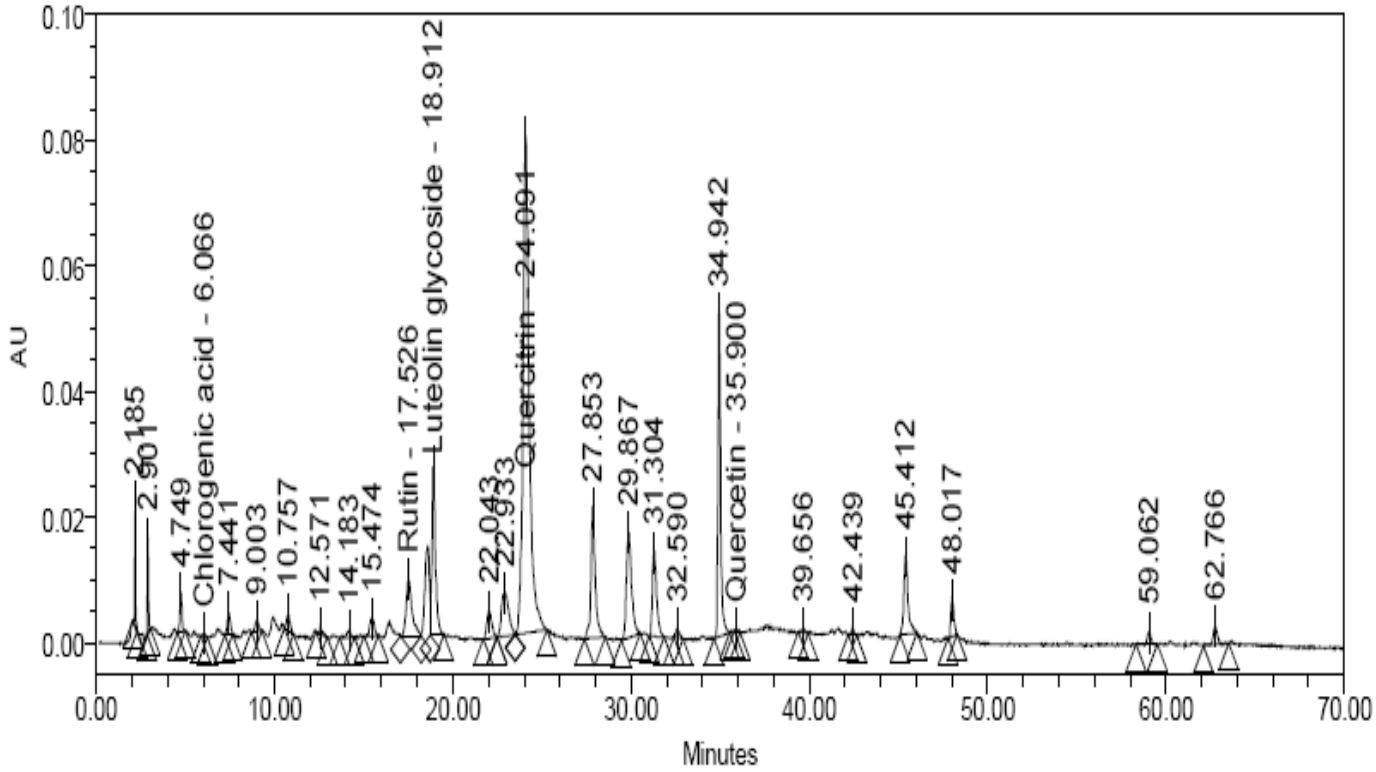

Figure 1: HPLC Chromatogram

	Peak Name	RT	Area (µV*sec)	% Area	Height (µV)	Amount	Units
1		2.185	53307	1.07	16475		
2		2.901	52753	1.06	14666		
3		4.749	49579	0.99	6214		
4	Chlorogenic Acid	6.066	5809	0.12	559	2.140	Ng
5		7.441	42348	0.85	3490		
6		9.003	24394	0.49	1774		
7		10.757	35552	0.71	2563		
8		12.571	10619	0.21	599		
9		14.183	17798	0.36	1009		
10		15.474	49023	0.98	2725		
11	Rutin	17.526	209016	4.18	8952	136.280	Ng
12	Luteolin glycoside	18.912	383353	7.67	26871	179.197	Ng
13		22.043	83067	1.66	4479		
14		22.933	197100	3.94	6704		
15	Quercitrin	24.091	1924734	38.50	79200	1069.306	Ng
16		27.853	348500	6.97	20575		
17		29.867	342464	6.85	16785		
18		31.304	227956	4.56	13458		
19		32.590	19788	0.40	1326		
20		34.942	549400	10.99	51792		
21	Quercetin	35.900	4153	0.08	479	1.978	Ng
22		39.656	7932	0.16	551		
23		42.439	10887	0.22	1161		
24		45.412	202231	4.05	12390		
25		48.017	69339	1.39	5784		
26		59.062	28691	0.57	1963		
27		62.766	49070	0.98	2968		

Table 2: Results of HPLC Chromatogram

Sr. No.	Sample Name	Rutin	Quercetin	Quercetrin	Luteolin glycoside	Chlorogenic acid
1.	*Euphorbia helioscopia* (aerial part) MeOH Extract	0.136	0.001	1.069	0.179	0.002

Table 3: Percentage of markers present in methanolic extract

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