# REPORT

# Physicochemical and phytochemical analysis of *Euphorbia helioscopia* (L.)

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**Abstract**: The present study aimed to standardize the crude drug from "*Euphorbia helioscopia*" by doing qualitative and quantitative analysis of different pulverized plant parts and extracts. Physicochemical analysis (determination of moisture contents, total ash, water insoluble ash, sulphated ash, acid insoluble ash, and water and alcohol extractives) was done on powdered raw materials (stem and leaves). The moisture contents and the ash value were found within the normal recommended range (moisture contents 6% and ash value 20%). The value of water-soluble extracts was higher as compared to alcohol soluble extractives. Percentage yield was highest in methanol solvent. The phytochemical analysis i.e. total lipids, total proteins and carbohydrates of crude powder showed that lipids and proteins contents were high (2.4% & 0.91% respectively) in pulverized stem while carbohydrate contents were high (78.27%) in pulverized leaves. Qualitative analysis by FTIR fingerprints and UV-scanning showed that stem and leaves of the plant contained the same constituents because their spectra are super-imposable. Aqueous-, ethanol-, petroleum ether-, chloroform- and methanol extracts were used in the study. Quantitative analysis was done by calculating the primary and secondary metabolites (total proteins, total glycosaponins, total alkaloids, total flavonoids, and total polyphenolics) in all the extracts using suitable markers. Chloroform gave very less percentage yield and nil primary metabolites so it was eliminated from secondary metabolites estimation. The maximum value of total proteins, total glycosaponins, total alkaloids, total flavonoids and total polyphenolics were found in the leaves methanol (36.56%), stem methanol (34%), stem ethanol (41.84%), leaves methanol (108.96%), and leaves petroleum ether (7.22%) respectively. Different pharmacological activities of the plants are due to their flavonoid contents. It is concluded that methanol is the best solvent for extraction. Any arial part of the plant can be used in pharmacological evaluations prior to pre-clinical and clinical studies because leaves and stem had superimposable spectra in FTIR and UV-scanning.

**Keywords**: Crude extract, *Euphorbia helioscopia*, primary and secondary metabolites, quantitative analysis, FTIR.

## INTRODUCTION

*Euphorbia helioscopia* L. *(Euphorbiaceae*) is found throughout the Punjab while grown in Nilgiri Hills (Nadkarni, 2002). *Euphorbiaceae* family comprised 8000 species which are used for the treatment of number of diseases such as warts cure, intestinal parasites, skin diseases, gonorrhea, migraine (Webster, 1994 and Kinghorn *et al*., 1975). Kinghorn *et al.* found plant lattice as insecticide, fish poison, and as ordeal poison. Traditionally leaves and stems of *Euphorbia helioscopia* L. are used for vermifuge and febrifuge actions (Wu *et al*., 1991), roosted pepper mixed with seeds are used in cholera , oil obtained from seeds used in constipation, and roots are used as anthelmintic (Panda, 2004). Additionally the plant has been used in studies for different pharmacological activities such as insulin secretagogue (Hussain *et al*., 2004), antibacterial (Uzair *et al*., 2009, Farhat *et al*., 2011 and Ramezani *et al.,* 2008), antifungal (Uzair *et al*., 2009 and Farhat *et al*., 2011), phytotoxicity (Uzair *et al*., 2009), vasodepressor (Barla *et al*., 2006), antiviral (Ramezani *et al.,* 2008), anticancer and or antitumor (Wang *et al.,* 2012), allelopathic (Tanveer *et al*., 2010), antioxidant (Uzair *et al*., 2009; Nikolova *et al*., 2011 and Ben-Mohamed *et al.,* 2012), anti-allergic and anti-asthmatic (Park *et al*., 2001), effect on breast cancer resistant protein ((BCRP) and P-glycoprotein (ABCB1 and ABCG2), (Barile *et al.,* 2008), cytotoxic (Zhang *et al.,* 2006), molluscicidal (Najia *et al*., 2000), cholinergic activity and brine shrimps toxicity (Chaudhry *et al*., 2001). The plant has great medicinal importance due to these pharmacological properties. The plant is rich in lead compounds that can serve for the development of new drugs. Standardization is imperative to get reproducible pharmacological activities and a best quality product. Herbal products standardization is always a tedious target due to variations in phytochemical constituents (due to

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variation in collection time, drying, storage and number of other factors) and unavailability or inadequacy of standards and methods of analysis. WHO recommended few guidelines, implementation of them can minimize these phytochemical variations (Wani *et al*., 2007). These variations can further be minimized by the application of pharmacognostic methods to know which plant, identified by botanical nomenclature and which part of the plant and what percentage is used. Physicochemical properties and phytochemical analysis of the raw material help in positive identification "that the herb is what it is claimed to be". Keeping in view the medicinal and commercial importance of *Euphorbia helioscopia*, the present study aimed to analyze crude powder and extracts of different parts of the plant for physiochemical properties and phytochemical analysis using Fourier Transform Infrared (FTIR) and UV spectroscopy.

## METHODOLOGY

***Collection of plant material, authentication and extraction***

Plant was collected from the suburbs of Lahore in the month of February, 2012. It was identified by a taxonomist of Botany Department, Government College University (GCU) Lahore-Pakistan. After authentication a voucher specimen (1501) was deposited in GCU Lahore herbarium. Stem and leaves were separated and dried under shade. After drying, they were ground individually. The powdered material was subjected to cold extraction (maceration) with water and ethanol and sequential extraction by using soxhlet in decreasing order of polarity with petroleum ether, chloroform, and methanol.

***Solvents***

Petroleum ether (Sigma Aldrich), ethanol (BDH Laboratory), chloroform and methanol (Merck, Germany), Folin-Ciocalteau reagent, potassium sodium tartrate, quercetin and piperine (Merck, Germany), BSA fraction V (Bioworld, bioplus fine research chemicals), sodium carbonate and potassium sodium tartrate (BDH chemical Ltd., England), sodium hydroxide, aluminium nitrate (Merck, Germany). gallic acid (China national chemical import and export corp. Shijiaz Huang Branch), triton (Uni-Chem Chemical Reagents) were purchased from local market. All the solvents and reagents were of analytical grade.

***Instruments***

Ultra-violet and visible spectrophotometer, UV- 1700 Pharma Spec (Shimadzu), FTIR spectrometer (Thermo Nicolet, USA) with OMNIC software (version 6.0 a) were used.

***Proximate analysis of powdered material***

*(a) Physicochemical analysis*

United States Pharmacopoeia-National Formulary (2003) method was adopted for studying physicochemical properties (extractive values, total ash, water and acid insoluble ash, sulphated ash and moisture contents).

***1-Moisture contents***

Powdered plant material (2g) was weighed in a tared china-dish. This china-dish was kept in oven for 30 minutes at 105°C for drying the plant material. After removing from oven, it was put in desiccator for cooling purpose. Then the cooled china-dish was weighed on digital balance and the weight of dried material was calculated by subtracting the empty china-dish weight from china-dish plus dried material weight. Moisture contents were calculated as follows:

Moisture contents = 100 - weight of dried material.

***2-Total ash***

Two grams powder plant material was weighed in a tared china-dish. Then it was incinerated in furnace at temperature 675 ± 25°C for the duration until ash got free from carbon. After getting desired form of ash, china-dish was placed in desiccator to cool its contents. At the end the ash contents were weighed and the percentage of total ash was calculated with reference to sample weight.

***3-Acid insoluble ash***

The total ash contents obtained from two grams of powdered plant material were boiled in 25 mL dilute HCl for 5 minutes. The boiled material was filtered through ash less filter paper. The soluble matter was collected as filtrate and insoluble material as residue on filter paper and it was washed with hot distilled water to ensure that all soluble material had been removed. This filter paper was then dried and ignited in tared china-dish for the time period until ash got free from carbon. Then it was cooled in desiccator. The ash contents were weighed and the percentage of acid insoluble ash was calculated with reference to weight of total ash used in test.

***4-Water insoluble ash***

The total ash contents obtained from two grams of powdered plant material were boiled in 25 mL distilled water for 5 minutes. The boiled material was filtered through ash less filter paper. The soluble matter was collected as filtrate and insoluble material as residue on filter paper and it was washed with hot distilled water to ensure that all soluble material had been removed. This filter paper was then dried and ignited in tared china-dish for the time period until ash got free from carbon. Then it was cooled in desiccator. The ash contents were weighed and the percentage of water insoluble ash was calculated with reference to weight of total ash used in test.

***5-Sulphated ash***

Sulphuric acid was mixed with two grams of powdered plant material in a tared china-dish to make a paste like material. This china-dish was ignited gently till white fumes stop originating from the surface of the material.

Then it was cooled in desiccator. The ash contents were weighed and the percentage of sulphated ash was calculated with reference to weight of dried powdered plant material used in test.

***6-Alcohol soluble extractives***

Five grams powdered plant material was put in tared flak. Ethanol 95% (100 mL) was poured on it for maceration. The sample was macerated in a closed flask for 24 hours with continuous stirring. The contents were filtered and 25 mL filtrate was evaporated to dryness in china dish and the residue was dried in oven at 105°C and weighed. The percentage of alcohol soluble extractives was calculated with reference to weight of sample.

***7-Water soluble extractives***

Five grams powdered plant material was put in tared flak. Ethanol 95% (100 mL) was poured on it for maceration. The sample was macerated in a closed flask for 24 hours with continuous stirring. The contents were filtered and 25 mL filtrate was evaporated to dryness in china dish and the residue was dried at 105°C in oven and weighed. The percentage of alcohol soluble extractives was calculated with reference to weight of sample.

***(b) Phytochemical analysis of powdered material***

The powdered plant material was analyzed to estimate primary metabolites, total lipids, total proteins (Lowry *et al*., 1951) and carbohydrates (Barminas *et al*., 1999 and Al-Hooti *et al*., 1998).

***1-Estimation of total lipids content***

Fifteen grams powder material was subjected to hot extraction using petroleum ether as solvent. Solvent macerated the sample in thimble for 12 hours and extraction was carried out for 24 hours. Temperature was maintained within the range of 40-60$^{o}$C during the extraction procedure. After 24 hours extraction, filtration was done and filtrate was dried in tared flask using rotary evaporator at 40$^{o}$C. After drying all the excess solvent, again flask was weighed and lipids content were calculated.

***2-Estimation of total proteins content***

One gram of powdered plant material was macerated for 10 hours with distilled water in which two-three drops of triton-X were added. After 10 hours, material was filtered and this filtrate was used in total protein contents estimation according to Lowry *et al*. (1951) modified method. Briefly, extract (10 mL) was centrifuged at 2700 rpm for 10 minutes. Then 0.1 mL of supernatant was taken in a test tube and the volume was made up to 1 ml with distilled water. Then 3 mL of reagent C [Reagent C was prepared by mixing 50 mL of reagent A and 1 mL of reagent B. Reagent A composed of 2% sodium carbonate in 0.1 N sodium hydroxide and reagent B was prepared by mixing 0.5% copper sulfate in 1% potassium sodium tartarate] and 0.2 ml of Folin-Ciocalteau reagent were added to this test tube and it was then incubated for 30 minutes at room temperature and absorbance was recorded at 600 nm. Bovine serum albumin [BSA] (Fraction V) solution was used as standard. Blank was prepared similarly containing all the reagents except sample. BSA was used in different concentration range in order to plot standard curve. Total proteins content were calculated from the standard curve using linear regression equation. The test was repeated in triplicate.

***3-Estimation of carbohydrates***

Carbohydrate contents were calculated by subtracting sum of percentages of moisture contents, total ash, total proteins and total lipids from 100 (Al-Hooti *et al.,* 1998 and Barminas *et al.,* 1999).

**(c) *Qualitative analysis of crude powders by FTIR spectroscopy***

The powdered stem and leaves of *Euphorbia helioscopia* were analyzed in triplicate to get FTIR spectra using KBr discs as follows: 01 mg of the crude drug powder and 100 mg KBr were ground together and the mixture was transferred to a die. The die was then pressed in hydraulic press to produce discs which were used to get FTIR spectra in mid IR range 4000-400 cm$^{-1}$. FTIR fingerprint profiles of different parts of the plant were compared with each other for comparison.

**(d) *Studies of extracts***

***I-Estimation of metabolites (primary and secondary)***

Extracts were analyzed for quantification of metabolites (primary and secondary) such as total proteins (Lowry *et al*., 1951), total polyphenols (Slinkard and Singleton, 1997), total flavonoids (Chang *et al*., 2002), total alkaloids and total glycosaponin (Anonymous, 2005).

***II-Estimation of primary metabolites***

***1-Estimation of total proteins***

Fifty mg extract was mixed with 10 mL distilled water in centrifuge tube. After vortex for 2 minutes, tube was centrifuged at 2700 rpm for 10 minutes. Then 0.1 mL of supernatant was taken in a test tube and the volume was made up to 1 ml with distilled water. Then 3 mL of reagent C [Reagent C was prepared by mixing 50 mL of reagent A and 1 mL of reagent B. Reagent A composed of 2% sodium carbonate in 0.1 N sodium hydroxide and reagent B was prepared by mixing 0.5% copper sulfate in 1% potassium sodium tartarate] and 0.2 ml of Folin-Ciocalteau reagent were added to this test tube and it was then incubated for 30 min at room temperature and absorbance was recorded at 600 nm. Bovine serum albumin [BSA] (Fraction V) solution was used as standard. Blank was prepared similarly containing all the reagents except sample. BSA was used in different concentration range in order to plot standard curve. Total proteins content were calculated from the standard curve

**Table 1**: Physicochemical analysis (%age)

| Samples (powder) | Moisture contents | Total ash contents | Water insoluble ash | Acid insoluble ash | Sulphated ash | Water soluble extractives | Alcohol soluble extractives |
|---|---|---|---|---|---|---|---|
| Stem | 6 | 14.25 | 28.57 | 10.71 | 19.5 | 34.96 | 16.72 |
| Leaves | 6 | 14.5 | 34.48 | 10.34 | 19 | 39.44 | 14 |

**Table 2**: Phytochemical analysis of raw material (%age)

| Crude Powder | Total Lipids Contents | Total Protein Contents | Carbohydrate Contents |
|---|---|---|---|
| Stem | 2.4 | 0.91 | 76.44 |
| Leaves | 0.4 | 0.83 | 78.27 |

**Table 3**: Percentage yield of various extracts

| | AQ | ET | PE | CH | MT |
|---|---|---|---|---|---|
| Stem | 8.74% | 4.18% | 5.00% | 1.45% | 13.98% |
| Leaves | 9.86% | 3.50% | 5.06% | 1.70% | 14.04% |

**Table 4a**: Phytochemical analysis of various extracts (primary metabolites)

| Extracts | Total proteins (%) | | Total Glycosaponins (%) | |
|---|---|---|---|---|
| | Mean | SD | Mean | SD |
| L.AQ | 14.31 | ± 1.042 | 06.3 | ± 0.0120 |
| L.ET | 24.17 | ± 1.117 | 13.4 | ± 0.1510 |
| S.AQ | 10.35 | ± 0.075 | 01.0 | ± 0.6520 |
| S.ET | 17.82 | ± 0.000 | 29.2 | ± 0.2310 |
| L.PE | 03.19 | ± 0.189 | 00.0 | ± 0.0000 |
| L.CH | 06.34 | ± 0.100 | 00.0 | ± 0.0000 |
| L.MT | 36.57 | ± 1.090 | 23.8 | ± 0.2520 |
| S.PE | 30.26 | ± 0.144 | 00.0 | ± 0.0000 |
| S.CH | 07.16 | ± 0.122 | 00.0 | ± 0.0000 |
| S.MT | 16.39 | ± 0.214 | 34.0 | ± 0.1020 |

**Table 4b**: Phytochemical analysis of various extracts (secondary metabolites)

| Extracts | Total alkaloids (%) | | Total Flavonoids (%) | | Total polyphenolics (%) | |
|---|---|---|---|---|---|---|
| | Mean | SD | Mean | SD | Mean | SD |
| L.AQ | 25.00 | ± 0.030 | 048.651 | ± 0.277 | 2.526 | ± 0.006 |
| L.ET | 21.27 | ± 0.017 | 051.825 | ± 0.000 | 4.225 | ± 0.015 |
| S.AQ | 19.79 | ± 0.030 | 044.683 | ± 0.277 | 2.572 | ± 0.017 |
| S.ET | 41.84 | ± 0.012 | 049.444 | ± 0.277 | 3.643 | ± 0.012 |
| L.PE | 36.98 | ± 0.025 | 055.000 | ± 0.728 | 7.228 | ± 0.015 |
| L.MT | 03.56 | ± 0.133 | 108.968 | ± 0.271 | 3.759 | ± 0.010 |
| S.PE | 13.02 | ± 0.025 | 060.556 | ± 0.277 | 2.735 | ± 0.006 |
| S.MT | 18.66 | ± 0.017 | 037.540 | ± 0.277 | 3.038 | ± 0.010 |

L=Leaves, S=Stem, AQ=Aqueous, ET=Ethanol, PE=Petroleum ether, CH=Chloroform, MT=Methanol

using linear regression equation. The test was repeated in triplicate.

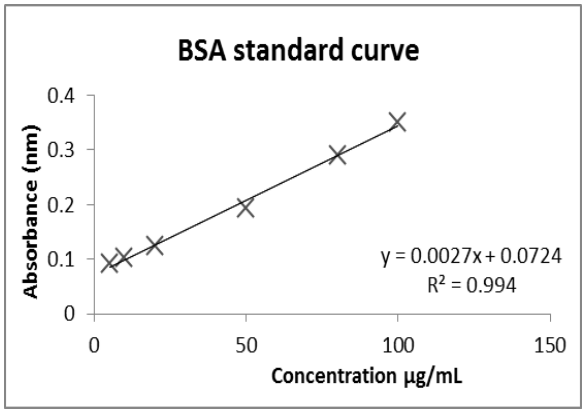
***2-Estimation of total glycosaponins***

One gram extract was dissolved in 50 mL methanol and refluxed for 30 minutes and filtered. Excessive methanol in filtrate was removed by rotary evaporator and the filtrate was concentrated to 10 mL. This concentrated extract was added drop wise to 50 mL acetone in a tared beaker. Then saponins precipitates appeared in the beaker. The precipitates were dried in oven at 100$^{o}$C to constant weight and glycosaponins were calculated by dividing the weight of precipitate with weight of extract and multiplied by 100. The test was repeated in triplicate.

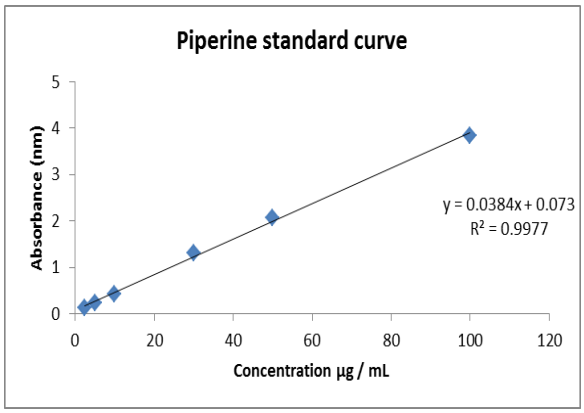
***III-Estimation of secondary metabolites***

***1-Estimation of total polyphenolics***

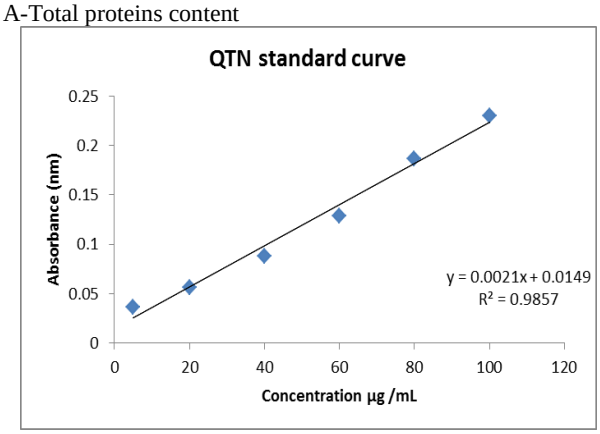
Singleton and Slinkard (1997), method with minor changes was used for phenolic contents determination. Gallic acid was used to draw the standard curve. Sample



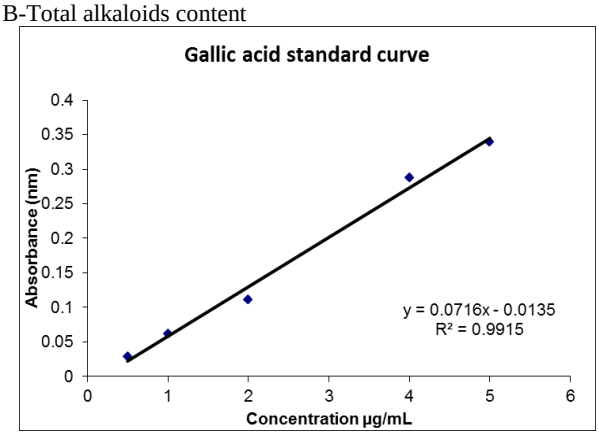

A-Total proteins content




B-Total alkaloids content




C-Total flavonoids content




D-Total polyphenolics content

**Fig. 1**: Standard curves of total protein, alkaloids, flavonoids and polyphenolic contents.

(0.2 mL) and standard (0.2 mL) were taken in test tubes and Folin-Ciocalteu's phenol reagent (0.2 mL) was added to these test tubes and mixed them thoroughly. One mL of $Na_2CO_3$ solution (15%) was added after 4 minutes. Then mixture was allowed to stand at room temperature for 2 hours and at 760 nm absorbance was recorded. Blank used contained all the reagents except analyte. Total polyphenolics contents were determined as mg of gallic acid equivalents by linear regression equation, obtained from gallic acid standard curve. The total polyphenolics contents were estimated using following equation.

Total phenols = Gallic acid equivalents (µg/mL) X extract volume / Sample (g). The test was repeated in triplicates.

***2-Estimation of total flavonoids***

Chang *et al.* (2002) method with little modifications was adopted. Quercetin (QTN) was used to draw the standard curve. Sample (0.2 mL) and standard (0.2 mL) were taken in test tubes and10% aluminum nitrate solution (0.1 mL), 1M potassium acetate (0.1 mL) and 4.6 mL distilled water were added to them. The test tubes were incubated at room temperature for 45 minutes. Blank was prepared similarly except analyte. Absorbance was measured at 415 nm. The flavonoid contents were determined as mg of quercetin equivalents by linear regression equation, obtained from the calibration curve of quercetin. Total flavonoids contents were calculated by following equation:

Total flavonoids = QTN equivalents (µg/mL) X extract volume / Sample (g). The test was repeated in triplicate.

***3-Estimation of total alkaloids***

Piperine was used to draw standard curve. Concentrated sulfuric acid (2 µL), alcoholic solution of the sample/ standard (100 µL), and 5% gallic acid solution in methanol (100 µL) were mixed in a test tube. The mixture was heated for 10 min in water bath and absorbance was measured at 660 nm against a blank containing equivalent amount of methanol in place of sample. Amide contents were calculated from linear regression, obtained from piperine standard curve. Total amide contents were expressed as mg equivalents of piperine and estimated by following equation: Total amides = Piperine equivalents (µg/mL) X extract volume / Sample (g).

## STATISTICAL ANALYSIS

All the samples and standards were analyzed in triplicate and results were expressed as mean ± SD. Primary and secondary metabolites in stem and leaves of the plant were analyzed by analysis of variance (One way ANOVA) to compare the means. P value < 0.05 was considered significant.

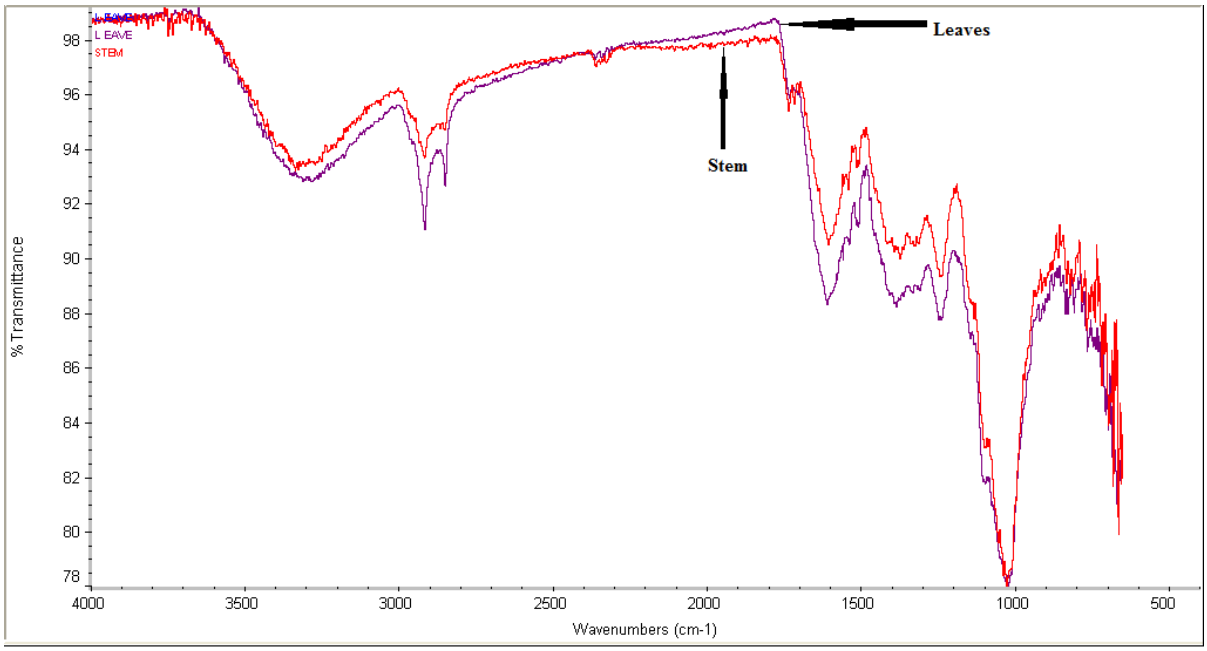



**Fig. 2**: FTIR spectrum of pulverized leaves and stem of *Euphorbia helioscopia* (L.)

## RESULTS

The results of physicochemical properties indicate that different parts of *Euphorbia helioscopia* (stem and leaves) have different content of moisture, total ash, acid and water insoluble ash, sulphated ash, alcohol soluble extractives and water soluble extractives (Table 1). The moisture contents and ash value were found within the normal recommended range (moisture contents 6% and ash value 20%). The value of water soluble extractives was higher as compared to alcohol soluble extractives. Highest percentage yield was achieved with methanol using soxhlet extraction (table 3). Phytochemical analysis of crude powder i.e. total lipids, proteins and carbohydrates are presented in table 2. It can be seen that lipids and proteins contents were high (2.4% & 0.91% respectively) in pulverized stem while carbohydrates contents were high (78.27%) in pulverized leaves (table 2).

The results of primary and secondary metabolites of extracts are presented in table 4a and 4b respectively. Total proteins, total alkaloids, total flavonoids and total polyphenolics were estimated using linear regression equation $y = 0.0027x + 0.0724$ ($R^2 = 0.994$) which was obtained from bovine serum albumin (fraction V) standard curve (Fig. 1A), $y = 0.0384x + 0.073$ ($R^2 = 0.9977$) which was obtained from piperine standard curve (fig. 1B), $y = 0.0021x + 0.0149$ ($R^2 = 0.9857$) which was obtained from quercetin (QTN) standard curve (fig. 1C) and $y = 0.0716x - 0.0135$ ($R^2 = 0.9915$) which was obtained from gallic acid standard curve (fig. 1D) respectively. Gravimetric assay was used for estimation of glycosaponins.

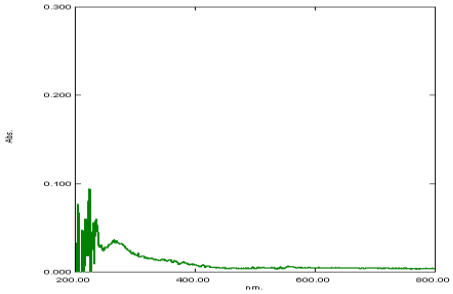
The distribution of total metabolic contents were in this order; Total Protein L.MT> L.ET> S.ET> S.MT> L.AQ> S.AQ> S.CH> L.CH> S.PE> L.PE; Glycosaponins S.MT> S.ET>L.MT> L.ET> L.AQ> S.AQ; Total Alkaloid S.ET> L.PE> L.AQ> L.ET> S.AQ> S.MT> S.PE>L.MT; Total Flavonoids L.MT> S.PE> L.PE> L.ET> S.ET> L.AQ> S.AQ> S.MT; Total Polyphenolics L.PE> L.ET>L.MT> S.ET> S.MT> S.PE> S.AQ> L.AQ.

FTIR spectrum of pulverized stem and leaves (fig. 2) and UV profile of aqueous, ethanol, petroleum ether, chloroform and methanol extracts of stem and leaves of *Euphorbia helioscopia* showed identical profile (fig. 3).

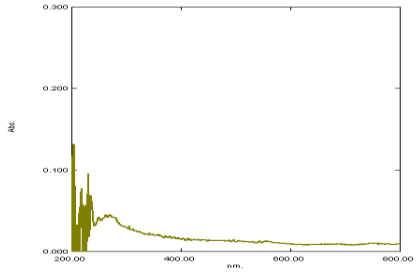
## DISCUSSION

The objective of physicochemical analysis (moisture contents, ash value and total extractives in methanol and water) and phytochemical analysis (total proteins, total lipids and carbohydrates found in crude drug and total proteins, total glycosaponins, total alkaloids, total flavonoids and total polyphenolics present in extracts) were to standardize the natural medicinal plant. Stability of natural products is greatly dependent on moisture contents. Less moisture content is needed for prevention of chemical decomposition and microbial contamination in the natural products. Moisture content is estimated by calculating the loss of weight (of crude powder) on drying in oven. This method is only applicable to those raw materials that contain non-volatile constituents. The

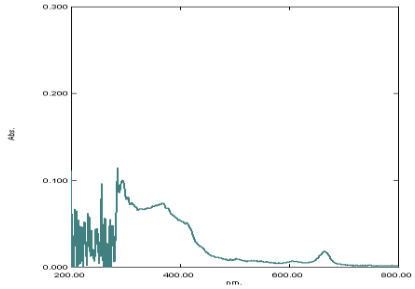
A-leaves aqueous



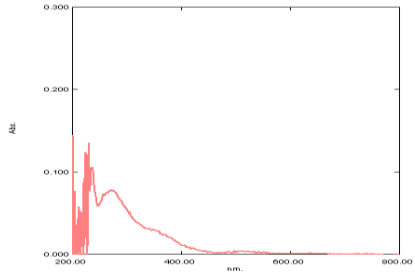
B-stem aqueous



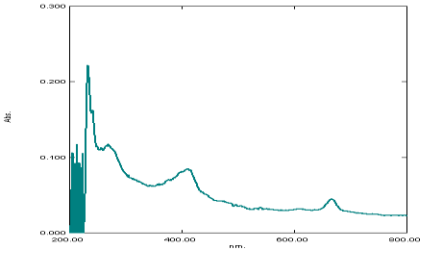
C-leaves ethanol



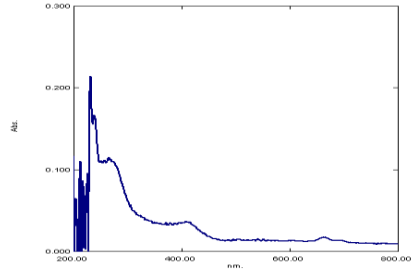
D-stem ethanol



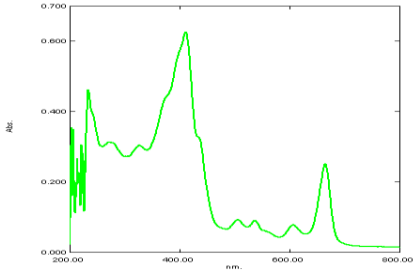
E-leaves petroleum ether



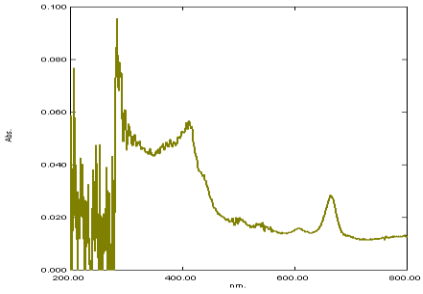
F-stem petroleum ether



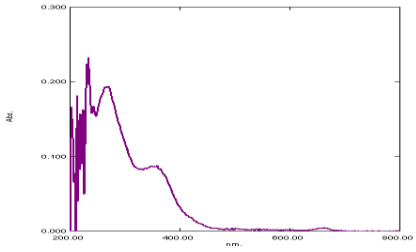
G-leaves chloroform



H-stem chloroform



I-leaves methanol



J-stem methanol

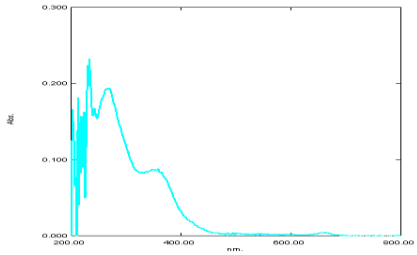


**Fig. 3**: UV-scanning plots of all the extracts.

quality and purity of powdered crude drugs are determined by estimating ash value. All the organic matter traces are removed during ash test; their presence may interfere in analysis. Crude drug on incineration normally leaves an ash consisting of carbonates, silicates and phosphates of sodium, calcium, potassium and

magnesium. The total ash of a crude drug determines that how much care is required in its preparation. Acid insoluble ash test is performed if the crude drug has silica or calcium oxalate. Sulphated ash is less fusible than the ordinary ash that's why some analysts add sulphuric acid with powdered crude drug prior to performing ash test (Genest *et al.*, 1963).

Methanol was found best solvent for extraction because it gave highest percentage yield and chloroform was poor solvent, giving very low yield. Primary metabolites were nil in chloroform extracts, thus secondary metabolites analysis was not performed on this solvent extracts. FTIR profile of powdered material (stem and leaves) and UV profile of different extracts of leaves and stem are identical, so it is concluded that any aerial part can be used for pharmacological evaluation because both parts contain same constituents.

FTIR profiles are important in quality assessment of herbal materials because often it is not necessary to know the identity of individual constituents that make up fingerprint. Moreover, FTIR spectroscopy is a non destructive technique and offers the analysis of plant material on solid matrix. Fingerprints, characteristic to each material, give quick check of plant material giving reliable indication of some identity or difference. The materials having similar constituent's exhibit similar UV spectra whish are superimposable. Materials having superimposable spectra may possess similar pharmacological activity.

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