

Original Research Article

Radical scavenging activity and cytotoxicity of *Euphorbia hirta* L. growing in Egypt

Riham Omar Bakr^{1*}, Mohamed Abd El Aziz El-Raey², Christien Nader Farouk¹, Hadil Emad Ahmed¹, Ola Mohamed El Said¹

*Corresponding author:

Riham Omar Bakr

¹Pharmacognosy Department, Faculty of Pharmacy, October University for Modern Sciences and Arts, Giza, Egypt

²Phytochemistry and Plant Chemosystematics Department, National Research Center, Dokki, Cairo, Egypt

Abstract

Euphorbia hirta L. (asthma weed, pill bearing spurge), is one of the most important species of Euphorbiaceae indigenous to Egypt and widely used in folk medicine. It was the subject for many phytochemical and biological studies but correlation between phytoconstituents and biological activities were not well studied. The aim of the present study was to correlate the antioxidant and cytotoxic activities against two of the most important health problems in Egypt with the main constituents in *E. hirta* (phenolic and flavonoid). The different fractions obtained from successive extraction of *E. hirta* were screened for their radical scavenging ability using 1,1-diphenyl-2-picrylhydrazine (DPPH) test in addition to cytotoxicity using Sulforhodamine B (SRB) assay against liver (Huh-7) and lung (A-549) cell carcinoma. Phenolic and flavonoid contents were estimated using colorimetric assays (Folin-Ciocalteu and aluminum chloride assays respectively). Ethyl acetate (EtOAc) fraction appeared as potent radical scavenger (IC₅₀ 5.4±0.65 µg/ml and IC₉₀ 11.9±0.84 µg/ml) and had the highest potency against Huh-7 (IC₅₀ 36.7±1.02 µg/ml) and A-549 (IC₅₀ 114.4 ±0.78 µg/ml) cancer cell lines. These results are attributed to the highest flavonoid concentration (23±1.06 mg/g quercetin equivalent) in EtOAc fraction in addition to high content of phenolics (88.9 ±0.57 mg/g gallic acid equivalent). Further studies are necessary to isolate and identify the ethyl acetate's bioactive compounds and evaluation of their biological activities.

Keywords: Cytotoxicity, DPPH, *Euphorbia hirta*, phenolic content, SRB assay.

Introduction

Medicinal plants have been used as a source of remedies since ancient times. The ancient Egyptians were familiar with many medicinal herbs and were aware of their usefulness in treatment of various diseases. Nowadays, there is an increasing trend towards the use of herbal medicine in Egypt that reflects an increasing confidence in such remedies. The flora of Egypt includes about 2000 species of plants distributed in its different localities that vary in type of soil and prevailing climatic and other environmental conditions that hence encourage the growth of a wide range of plant species [1].

The Euphorbiaceae is a huge flowering family comprising 300 genera and 8000 species and it's widely distributed in tropical Africa. This family consists of species of great economic importance like *Ricinus communis*, *Manihot esculenta*, *Hevea brasiliensis*, *Euphorbia pulcherrima*, *Euphorbia esula* and *Barbados nut*. It contains different classes of active constituents as terpenoids, phenolic, flavonoids and alkaloids [2].

The genus *Euphorbia* is the biggest genus in Euphorbiaceae represented in the Egyptian flora by 42 species [3]. *Euphorbia hirta* is widely distributed in Egypt, in addition to tropical and subtropical regions of the world. The flowers are monoecious in

shape, leaves are opposite, narrowed ovate or oblong and its stipule are minute, linear and hairy. It is characterized by a cyme inflorescence with short rachis. It has one ovary tricarpedal. The fruits are characterized by their attractive yellow color, three-celled and the seeds are ovate however quadrangular in cross section, with band on each facet [4].

E. hirta was traditionally used in respiratory system disorders, such as laryngeal spasms, emphysema, asthma, bronchitis, hay fever, coughs, common cold [5], menstrual cycle disorders, kidney stones, sterility and sexually transmitted diseases [6].

E. hirta was the subject for many phytochemical studies reporting the isolation and identification of flavonoids such as quercetin, myricitrin, afzelin, scopoletin, scoparone, isoscapoletin, quercetin, isorhamnetin, pinocembrin, kaempferol and luteolin [7, 8], phenolic (gallic acid, 3,4-di-O-galloylquinic acid, 2,4,6-tri-O-galloyl-D-glucose and 1,2,3,4, 6-penta-O-galloyl-beta-D-glucose) [9], tannin content such as euphorbins A, B, C, O, E, 7, triterpenes and phytosterols such as β-amyirin, 24-methylenecycloartenol, and β-sitosterol, in addition to alkanes which include heptacosane, nonacosane and others. 3, 7, 11, 15-tetramethyl-2-hexadecen-1-ol, 6,10,14-trimethyl-2-pentadecanone, hexadecanal, phytol and n-

hexadecanoic acid (1'R, 5'A)-5-(5-methylcarboxymethyl-2-oxocyclopentyl)-3Z-pentenyl-β-D-(6-O-p-hydroxybenzoyl) glucopyranoside were also reported [10].

To build scientific basis for traditional uses of *E. hirta*, it was the subject for many biological studies. *E. hirta* showed proliferation inhibition of *Plasmodium falciparum* in addition to exhibiting minute cytotoxic property against human epidermis carcinoma KB 3-1 cells [5], sedative [11], anti-inflammatory [12, 13], anti-diarrheal activity [14, 15], antiasthmatic [16], antibacterial effect [17-21], radical scavenger [22-24], in addition to antiproliferative effect on Hep-2 cells from human epithelioma of larynx [25].

In this study the radical scavenging activity of *E. hirta* was investigated using DPPH assay in addition to cytotoxic activity estimated using SRB assay against liver (Huh-7) and lung (A-549) cancer cell lines. Phenolic and flavonoid contents have been estimated using colorimetric assays (Folin-Ciocalteu and aluminum chloride assays respectively).

Material and methods

Plant material

Euphorbia hirta L. was collected from Ismailia road (Obour town), Egypt and identified by Dr. Mona M. Marzouk (Department of Phytochemistry and Chemotaxonomy). A voucher specimen (RS007) was deposited in the herbarium of Pharmacognosy department, Faculty of Pharmacy, MSA University.

Chemicals

All chemicals used, including solvents, were of analytical grade. DPPH, folin-ciocalteu's phenol reagent, quercetin, and gallic acid were purchased from (Sigma-Aldrich Chemie, Steinheim, Germany).

Preparation of plant extract and successive fractions

The powdered air-dried aerial parts of *E. hirta* (1 kg) were exhaustively extracted with 70% methanol. The combined extracts were evaporated under vacuum. The residue was weighed and suspended in water, then exhaustively defatted with petroleum ether (60 to 80 °C) (Pet. ether). Combined Pet. ether subextracts were evaporated under reduced pressure (yield: 3.5%). Methanol was removed from the remaining extract and diluted with distilled H₂O to 400 ml and successively extracted with chloroform (Cf) (20 500 ml) and Ethyl acetate (EtOAc) (20 500 ml). Each solvent extract was evaporated to dryness under reduced pressure to give Cf (yield: 1.3%) and EtOAc (yield: 3.7 %), respectively. The remaining aqueous extract was further extracted with n-butanol (BuOH) (20 500 ml) and evaporated to dryness to yield BuOH (yield: 2.1%). The final aqueous phase was also evaporated to dryness (yield: 31.5%).

Biological activity

Radical scavenging activity

1,1-diphenyl-2-picryl hydrazine (DPPH) radical scavenging ability of the different fractions was evaluated using the method of Shimada *et al.* [26]. All plant fractions were screened at 100 µg/ml while the most potent fractions (giving more than 90%) were assayed at different concentrations to estimate IC₅₀. Methanolic solution of DPPH* (0.1 mM) was prepared; 1 ml of this solution was added to 3 ml of each sample solution at different conc. (0-75 µg/ml). The mixture was shaken vigorously and allowed to stand at room temperature for 30 min. Then the absorbance was measured at 517 nm (UV spectrophotometer Shimadzu 1800 UV probe). This method is based on the reduction of DPPH in methanol solution (dark purple color) in the presence of a hydrogen donating antioxidant due to the formation of the nonradical form DPPH-H in the reaction (lighter color) [27] so they scavenge free radical form of DPPH as shown in the following equation.



The DPPH scavenging effect and the decrease in absorbance was measured by the following equation:

$$DPPH \text{ scavenging } \% = 100 [(A_0 - A_1) / A_0] \times 100$$

Where A₀ was the absorbance of the control reaction and A₁ was the absorbance in the presence of the sample [28].

Cytotoxic assay

The cytotoxicity of each fraction was tested against liver (Huh-7) and lung (A-549) cancer cell lines by Sulforhodamine B (SRB) assay [29]. Exponentially growing cells were collected using 0.25% Trypsin-EDTA and plated in 96-well plates at 1000-2000 cells/well. Cells were exposed to test compounds for 72 h and subsequently fixed with trichloroacetic acid (TCA) (10%) for 1 h at 4 °C. After several washings, cells were exposed to 0.4% SRB solution for 10 min in dark place and subsequently washed with 1% glacial acetic acid. After drying overnight, Tris-HCl was used to dissolve the SRB-stained cells and color intensity was measured at 540 nm. The dose response curve of compounds was analyzed using Emax model.

$$\% \text{ Cell viability} = (100 - R) \times \left(1 - \frac{[D]^m}{K_d^m + [D]^m} \right) + R$$

Where R is the residual unaffected fraction (the resistance fraction), [D] is the drug concentration used, K_d is the drug concentration that produces a 50% reduction of the maximum inhibition rate and m is a Hill-type coefficient. IC₅₀ was defined as the drug concentration required to reduce fluorescence to 50% of

that of the control (i.e., $K_d = IC_{50}$ when $R=0$ and $E_{max} = 100-R$) [30].

Phytochemical screening

Phytochemical screening was performed using standard procedures [31, 32].

Phenolic and flavonoid contents

The Folin-Ciocalteu colorimetric method was used to measure the total phenolic (TP) content [33, 34]. Sample of 500 μ l of different fractions was diluted with 500 μ l distilled water then oxidized with 5ml of 0.2 N Folin-Ciocalteu reagent (FCR) and then the reaction was neutralized with 4 ml of the saturated sodium carbonate (75 g/L). The absorbance of the resulting blue color was measured at 765 nm with a spectrophotometer after incubation for 2 h at room temperature. Quantification was done on the basis of the standard curve of gallic acid (50, 100, 200, 300, 400 and 500 μ g/ml). Results were expressed as milligram of gallic acid equivalent (mg GAE) per gram dry extract.

Aluminium chloride colorimetric assay was used to measure the total flavonoid (TF) content [34, 35]. 0.5 ml was mixed with 1.5 ml 95% ethanol (v/v), 0.1 ml 10% aluminum chloride (w/v), 0.1 ml of 1 mol/L potassium acetate and 2.8 mL water. The volume of aluminum chloride was substituted by the same volume of distilled water in blank. After incubation at room temperature for 30 minutes, the absorbance of the reaction mixture was measured at 415 nm. Quantification was done using the quercetin (12.5, 25, 50, 80 and 100 μ g/ml) as standard and the results were expressed as milligrams of quercetin equivalent (mg QE) per gram dry extract.

Statistical analysis

Data are presented as mean \pm SD obtained upon three independent analyses.

Results

Radical scavenging activity

Comparing the DPPH free radical scavenging activity of total extract with different fractions of *E. hirta* indicated potential activity. EtOAc showed the lowest IC_{50} ($5.4 \pm 0.65 \mu$ g/ml) and IC_{90} ($11.9 \pm 0.84 \mu$ g/ml) followed by Cf with $IC_{50} 25.5 \pm 2.4 \mu$ g/ml and $IC_{90} 49.3 \pm 1.35 \mu$ g/ml (Figure 1) compared with vitamin C ($IC_{50} 42.80 \pm 1.5$) and butylated hydroxyanisole ($IC_{50} 53.9 \pm 3.1 \mu$ g/ml). On the other hand, pet. ether fraction showed the least activity.

Cytotoxic assay

SRB assay was used to evaluate the cytotoxicity pattern of different fractions in a dose dependent manner on two human cells carcinoma; liver (Huh-7) and lung cancer cell line (A-549). IC_{50} was measured for the different fractions and the percentage of surviving fraction was estimated (Table 1).

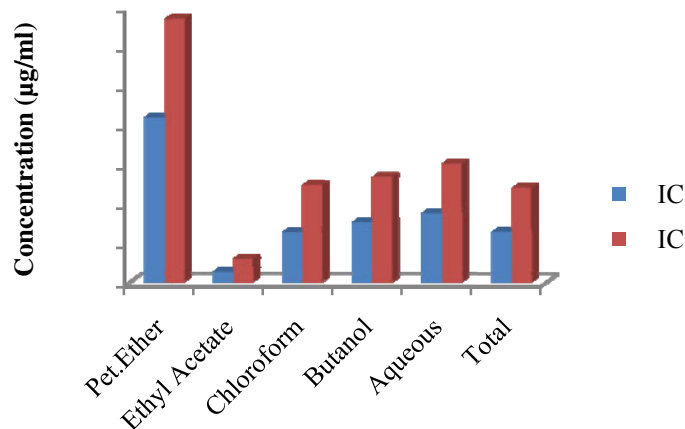


Figure 1: IC_{50} and IC_{90} of different fractions of *E. hirta* denoting antioxidant activity.

EtOAc fraction exhibited the highest potency against Huh-7 ($IC_{50} 36.7 \pm 1.02 \mu$ g/ml) followed by Cf ($IC_{50} 73.3 \pm 0.96 \mu$ g/ml). The resistant fraction of Huh-7 was 0% which denoted the potency of all fractions on liver cell carcinoma. In addition, EtOAc fraction showed the best effect against A-549 although with high IC_{50} ($114.4 \pm 0.78 \mu$ g/ml) followed by Cf ($IC_{50} 122.7 \pm 1.06 \mu$ g/ml) while the least potent is the aqueous fraction ($IC_{50} 1046 \pm 2.75 \mu$ g/ml) (Table 1). Substantial R-fraction of A-549 ranged from 9.5-10.6% which indicate the partial resistance of lung cell carcinoma.

Table 1: Cytotoxicity of *E. hirta* fractions against different solid tumor cell lines.

<i>E. hirta</i> fraction	Liver Cancer Cell Line (Huh7)		Lung Cancer Cell Line (A-549)	
	IC_{50} (μ g/ml)*	R %	IC_{50} (μ g/ml)*	R%
Ethyl Acetate	36.7 ± 1.02	0.0%	114.4 ± 0.78	10.6%
Petroleum Ether	107.5 ± 2.04	0.0%	148.3 ± 0.83	7.7%
Chloroform	73.3 ± 0.96	0.0%	122.7 ± 1.06	9.5%
Butanol	588 ± 2.75	0.0%	689.1 ± 2.43	-%
Aqueous	942 ± 2.98	0.0%	1046 ± 2.75	-%
Total	675 ± 3.12	0.0%	875 ± 2.21	-%

*Mean of triplicate determinations \pm SD

Phytochemical screening

Phytochemical screening of different fractions of *E. hirta* revealed the presence of tannins and flavonoids in considerable amount in Cf, EtOAc, BuOH and aqueous fractions while traces were found in pet. ether fraction. Sterols are present in considerable amounts in pet. ether, Cf and BuOH fractions and absent in EtOAc fraction. Anthraquinone was only noticed in Cf fraction while alkaloid and saponins are absent in all fractions.

Phenolic and flavonoid contents

Contents of TF were estimated by FCR in terms of gallic acid equivalent (standard curve equation: $y = 0.0011x + 0.0009$, $r^2 = 0.9867$). The TP ranged from 40.97 ± 0.34 to 391.4 ± 2.34 mg/g GAE (Table 2). Cfraction showed the highest phenolic concentration (391.4 ± 2.34 mg/g GAE) followed by BuOH (110.9 ± 1.63 mg/g GAE), then EtOAc (88.9 ± 0.57 mg/g GAE) and finally aqueous fraction.

Total flavonoid content was estimated by aluminium chloride colorimetric technique in term of quercetin equivalent (the standard curve equation: $y = 0.005x - 0.0198$, $r^2 = 0.9774$) ranged between 3.35 ± 0.82 to 23 ± 1.06 mg/g QE (Table 2). EtOAc fraction showed the highest concentration (23 ± 1.06 mg/g QE) followed by BuOH (9.24 ± 0.95 mg/g QE).

Table 2: Flavonoid and phenolic contents in *E. hirta* fractions.

<i>E. hirta</i> Extract	Total Phenolic (mg/g GAE)*	Total Flavonoid (mg/g QE)*
Chloroform	391.4 ± 2.34	4.6 ± 0.37
Butanol	110.9 ± 1.63	9.24 ± 0.95
Ethyl Acetate	88.9 ± 0.57	23 ± 1.06
Aqueous	40.97 ± 0.34	3.35 ± 0.82
Total	49 ± 0.86	7.55 ± 0.5

*Mean of triplicate determinations \pm SD

Discussion

Isolation of pure, pharmacologically active constituents from plants remains a long and tedious process. For this reason, it is necessary to develop methods able to eradicate needless separation procedures. Bioassay guided fractionation technique represent an important preliminary step for isolation of bioactive compounds where chemical screening is performed to allow localization and targeted isolation of new or useful constituents with potential activities [36]. Natural antioxidants are preferred in allopathic drugs to overcome the side effects. Most of the polar compounds such as phenolic and flavonoid substances are potent inhibitors of reactive oxygen species [37].

Cancer is a multi-step disease incorporating environmental, chemical, physical, metabolic, and genetic factors which play a direct and/or indirect role in the induction and deterioration of cancers. Strong and consistent epidemiology evidence indicates that a diet with high consumption of antioxidant rich fruits and vegetables significantly reduces the risk of many cancers, suggesting that certain dietary antioxidants could be effective agents for the prevention of cancer incidence and mortality. These agents present in the diet are a very promising group of

compounds because of their safety, low toxicity, and general acceptance [38].

DPPH* radicals have been used extensively as stable radicals to preliminarily evaluate the antioxidant activities of plant extracts. When testing the different fractions of *E. hirta* for their DPPH free radical scavenging activity, EtOAc fraction showed strong radical scavenging activity with the lowest IC_{50} followed by Cf. These results were in agreement with those of Kandalbhar et al [39].

SRB assay is a drug-induced cytotoxicity and cell proliferation test for large-scale drug-screening applications. Its principle is based on the ability of the protein dye (SRB) to bind electrostatically and pH dependent on protein basic amino acid residues of trichloroacetic acid-fixed cells. Under mild acidic conditions, it binds to and under mild basic conditions it can be extracted from cells and solubilized for measurement. The amount of luminescence is directly proportional to the number of living cells in culture [40].

Estimating the cytotoxic activity against Huh-7 and A-549, EtOAc fraction also exhibited the highest potency followed by Cf. These results are satisfying, contrary to Sidamaram et al [25] who tested the crude methanolic extract of *E. hirta* against HEP2 cells of human epithelioma of larynx using (3-(4, 5-dimethyl thiazol-2yl)-2, 5-diphenyl tetrazolium bromide (MTT) where IC_{50} was at concentration $625 \mu\text{g/ml}$. EtOAc fraction in this study showed higher potency against both cell lines used at lower concentration. Comparing the results of the current study with a previous study performed on *E. wallichii*, EtOAc showed the best radical scavenging activity using DPPH assay while when estimating the cytotoxic activity against lung carcinoma (H-157) and malignant melanoma (HT-144) BuOH fraction showed higher potency [41].

These results suggested that *E. hirta* contain compounds able to donate electron/hydrogen easily and having cytotoxic activity as polyphenolics. Phytochemical screening showed the abundance of tannins and flavonoids consistent with different reports while alkaloids are absent contrary to Patil and Magdum [42]. In a trial to quantify phenolic and flavonoid contents of *E. hirta*, traditional spectrophotometric assays were used to provide simple and fast screening methods. FCR was used to estimate TP using gallic acid as standard being the most abundant and important phenolic in natural products. EtOAc fraction exhibited high concentration while Cf fraction which followed EtOAc in its activity showed the highest concentration of phenolic content. Upon evaluating TF content using quercetin as standard, the highest flavonoid concentration was observed with EtOAc fraction followed by BuOH, aqueous and finally Cf fraction. A high degree of correlation was found between the radical scavenging activity (expressed as $1/IC_{50}$) and the flavonoid content ($r^2 = 0.86$) while this correlation was nearly absent with phenolic content ($r^2 = 0.001$). It is known that only flavonoids with a certain structure and particularly hydroxyl position in the molecule can act as proton donating and show radical scavenging activity [43]. Therefore, the strongest antioxidant activity and the highest cytotoxicity of EtOAc are attributed to great extent to the highest flavonoid concentration in *E. hirta*.

Conclusion

Ethyl acetate fraction of *E. hirta* revealed itself as a powerful radical scavenger and potent cytotoxic against liver carcinoma. Therefore further studies are necessary to isolate and identify its bioactive compounds correlated with its biological activities.

Author's contributions

CN Farouk, HE Ahmed and OM El Said prepared the plant extract, successive extraction and carried out phytochemical constituents screening. RO Bakr and MA El Raey have equal contribution in

carrying out biological screening, quantitative determinations and preparation of manuscript.

Acknowledgment

The authors are thankful to October University for Modern Sciences and Arts (MSA) and the National Research Center (NRC) for providing the necessary facilities.

Conflict of interest

The authors declare there are no conflicts of interest.

References

- [1]. Hagagg MY. Herbal Medicine in Egypt. In: Heywood VH, Skoula M editors. Identification of wild food and non-food plants of the Mediterranean region. Chania : CIHEAM-IAMC. 1997; p. 45- 55. Regional Workshop of the MEDUSA Network "Identification and use of Wild Plants of the Mediterranean Region", 1996;06:28-29, Chania (Greece).
- [2]. Radcliffe-Smith A. Genera Euphorbiacearum. 2001. Kew, UK. Royal Botanic Garden.
- [3]. Hegazi AM, Afifi MY, El Shorbagy MA, Elwan AA, El-Demerdashe, S. Egyptian National Action Program to Combat Desertification. Arab Republic of Egypt. Ministry of Agriculture and Land Reclamation Desert Research Center (DRC). 2005
- [4]. Bown D. Morphological characters of *E. hirta*. Encyclopaedia of Herbs and their Uses. 2005; 42 (2): 6-14.
- [5]. Tona L, Kambu K, Ngimbi N, Mesia K, Penge O, Lusakibanza M, Cimanga K, De Bruyne T, Apers S, Totte J, Pieters L, Vlietinck AJ. Antiamoebic and spasmolytic activities of extracts from some antidiarrheal traditional preparations used in Kinshasa, Congo. Phytomedicine. 2000; 7(1): 31-38.
- [6]. Singh SK, Yadav RP, Tiwari S, Singh A. Toxic effect of stem bark and leaf of *Euphorbia hirta* plant against freshwater vector snail *Lymnaea acuminata*. Chemosphere. 2005;59(2): 263-70.
- [7]. Liu Y, Murakami N, Ji H, Abreu P, Zhang S. Antimalarial flavonol glycosides from *Euphorbia hirta*. Pharmaceutical Biology. 2007;45(4): 278-281.
- [8]. Wu Y, Qu W, Geng D, Liang J-Y, Luo YL. Phenols and flavonoids from the aerial part of *Euphorbia hirta*. Chinese Journal of Natural Medicines. 2012; 10(1): 40-42.
- [9]. Chen L. Polyphenols from leaves of *Euphorbia hirta* L. Zhongguo Zhong Yao Za Zhi. 1991; 16 (1): 38-9.
- [10]. Ogunlesi M, Okiei W, Ofor E, Osibote AE. Analysis of the essential oil from the dried leaves of *Euphorbia hirta* Linn. (Euphorbiaceae), a potential medication for asthma. African Journal of Biotechnology. 2009; 8(24):7042-7050.
- [11]. Lanhers MC, Fleurentin J, Cabalion P, Rolland A, Dorfman P, Misslin R, Pelt J-M. Behavioral effects of *Euphorbia hirta* L.: sedative and anxiolytic properties. Journal of Ethnopharmacology. 1990; 29:189-98.
- [12]. Lanhers MC, Fleurentin J, Dorfman P, Mortier F, Pelt JM. Analgesic, antipyretic and anti-inflammatory properties of *Euphorbia hirta*. Planta Medica. 1991; 57(3):225-31.
- [13]. Daud MNH. Phytochemical analysis and biological activity of *Euphorbia hirta* and *Phyllanthus watsonii*. Master thesis, Faculty of Bioscience, Universiti Putra Malaysia, Malaysia. (2006)
- [14]. Galvez J, Zarzuelo A, Crespo ME, Lorente MD, Ocente MA, Jiménez J. Antidiarrhoeic activity of *Euphorbia hirta* extract and isolation of an active flavonoid constituent. Planta Medica. 1993; 59(4):333-336.
- [15]. Galvez J, Crespo ME, Jimenez J, Suarez A, Zarzuelo A. Anti-diarrhoeic activity of quercetin in mice and rats. Journal of Pharmaceutical Pharmacology. 1993; 45:157-159.
- [16]. Chopra RN, Chopra IC, Handa KL, Kapur LD. Chopra's Indigenous drugs of India. 2nd edition. Academic Publishers, Calcutta, India; 1994.
- [17]. Suresh K, Deepa P, Harisaranraj R, Achudhan V. Antimicrobial and phytochemical investigation of the Leaves of *Carica papaya* L., *Cynodon dactylon* (L.) Pers., *Euphorbia hirta* L., *Melia azedarach* L. and *Psidium guajava* L. Ethnobotanical Leaflets. 2008; 12: 1184-1191.
- [18]. Vijaya K, Ananthan S, Nalini R. Antibacterial effect of the aflavin, polyphenon 60 (*Camellia sinensis*) and *Euphorbia hirta* on *shigella* spp. a cell culture study. Journal of Ethnopharmacology. 1995; 49 (2):115-118.
- [19]. Mohamed S, Saka S, EL-Sharkawy SH, Ali AM, Muid S. Antimycotic

- screening of 58 Malaysian plants against plant pathogens. *Pesticide Sciences*. 1996; 47 (3):259–264.
- [20]. Kumar GS, Jayaveera KN, Kumar CK, Sanjay UP, Swamy BM, Kumar DV. Antimicrobial effects of Indian medicinal plants against acne-inducing bacteria. *Tropical journal of pharmaceutical research*. 2007;6 (2): 717-723.
- [21]. Olabiyi TI, Oyedunmade EEA, Ibikunle GJ, Ojo OA, Adesina GO, Adelasoye KA, Ogunniran TA. Chemical composition and bio-nematicidal potential of some weed extracts on *Meloidogyne incognita* under laboratory conditions. *Plant Sciences Research*. 2008;1 (2):30-35.
- [22]. Sharma NK, Dey S, Prasad R. *In vitro* antioxidant potential evaluation of *Euphorbia hirta* L. *Pharmacology Online*. 2007; 1:91–98
- [23]. Kumar S, Malhotra R, Kumar D. Antidiabetic and free radicals scavenging potential of *Euphorbia hirta* flower extract. *Indian Journal of Pharmaceutical Sciences*. 2010; 72 (4): 533–537.
- [24]. Kumar S, Malhotra R, Kumar D. *Euphorbia hirta*. Its chemistry, traditional and medicinal uses, and pharmacological activities. *Pharmacognosy Review*. 2010; 4 (7):58-61.
- [25]. Sidambaram R, Dinesh MG, Jayalakshmi ET. An *in vitro* study of cytotoxic activity of *Euphorbia hirta* on HEP-2 cells of human epithelioma of larynx. *International Journal of Pharmacy and Pharmaceutical Sciences*. 2011;3 (3):101-103.
- [26]. Shimada K, Fujikawa K, Yahara K, Nakamura T. Antioxidative properties of xanthan on the autooxidation of soybean oil in cyclodextrin emulsion. *Journal of Agricultural and Food Chemistry*. 1992; 40 (6):945–948.
- [27]. Gülçin I, Oktay M, Kireççi E, Küfrevioğlu ÖI. Screening of antioxidant and antimicrobial activities of anise (*Pimpinella anisum* L.) seed extracts. *Food Chemistry* 2003; 83 (3): 371-382.
- [28]. Oktay M, Gülçin I, Küfrevioğlu I. Determination of *in vitro* antioxidant activity of fennel (*Foeniculum vulgare*) seed extracts. *Food Science and Technology*. 2003; 36 (2):263–271.
- [29]. Skehan P, Storeng R, Scudiero D, Monks A, McMahon J, Vistica D, Warren JT, Bokesch H, Kenney S, and Boyd MR. New colorimetric cytotoxicity assay for anticancer-drug screening. *Journal of National Cancer Institution*. 1990; 82:1107-1112.
- [30]. Al-AbdAM, Lee JH, Kim SY, Kun N, Kuh HJ. Novel application of multicellular layers culture for *in situ* evaluation of cytotoxicity and penetration of paclitaxel 2008; *Cancer Sciences*. 99 (2):423-431.
- [31]. Harborne JB. *Phytochemistry* 1993. Academic Press, London, pp. 89-131.
- [32]. Trease GE, Evans WC. (2002). *Pharmacognosy*. 15th Edn. Saunders Publishers, London. Sellappan S, Akoh CC, Krewer G. Phenolic compounds and antioxidant capacity of Georgia-grown blueberries and blackberries. *Journal of Agricultural and Food Chemistry*. 2002; 50 (8), 2432-38.
- [33]. Bao J, Cai Y, Sun M, Wang G, Corke H. Anthocyanins, flavonols, and free radical scavenging activity of Chinese bayberry (*Myrica rubra*) extracts and their color properties and stability. *Journal of Agricultural and Food Chemistry*. 2005; 53 (6): 2327-2332.
- [34]. Kosalec, Bakmaz M, Pepeljnjak S, Vladimir-Knezevic, S. Quantitative analysis of the flavonoids in raw propolis from northern Croatia. *Acta Pharmaceutica*. 2004; 54, 65–72.
- [35]. Solis PN, Wright CW, Anderson MM, Gupta MP, Philipson JD. A microwell cytotoxicity assay using *Artemiasalina* (Brine shrimps). *Planta Medica*. 1993; 59 (3): 250 - 252.
- [36]. Owen RW, Haubner R, Mier W, Giacosa A, Hull WE, Spiegelhalder B et al. Isolation, structure elucidation and antioxidant potential of the major phenolic and flavonoid compounds in brined olive drupes. *Food and Chemical Toxicology*. 2003; 41(5): 703-717.
- [37]. Fresco P, Borges F, Diniz C, Marques MP. New insights on the anticancer properties of dietary polyphenols. *Medicinal Research Reviews*. 2006; 26 (6): 747-766.
- [38]. Kandalkar A, Manjunath KP, Patel A. (2011). Isolation and Antioxidant Potential of *Euphorbia hirta* Linn: Present Study related with the Antioxidant Activity of *Euphorbia hirta* Linn. leaves and also the isolation of Bioactive compound Myricitrin. LAP Lambert Academic Publishing. ISBN-13: 978-3-8443-1066-5.
- [39]. Voigt W. Sulforhodamine B assay and chemosensitivity. *Methods in Molecular Medicine*. 2005; 110: 39-48.
- [40]. Ul-Haq I, Ullaha N, Bibi G, Kanwal S, Ahmad MS and Mirza B. Antioxidant and cytotoxic activities and phytochemical analysis of *Euphorbia wallichii* root extract and its fractions. *Iranian Journal of Pharmaceutical Research*. 2012; 11 (1): 241-249.
- [41]. Patil SB and Magdum CS. Determination of LC50 values of extracts of *Euphorbia hirta* Linn and *Euphorbianeriifolia* Linn using brine shrimp lethality assay. *Asian Journal Research of Pharmaceutical Sciences*. 2011; 1(2):42-43.
- [42]. Mensor LL, Menezes FS, Leitao GG, Reis AS, dos Santos TC, Coube CS and Leitao SG. Screening of Brazilian plant extracts for antioxidant activity by the use of DPPH free radical method. *Phytotherapy Research*. 2001; 15: 127-130.