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PHYTOCHEMICAL ANALYSIS AND *IN-VITRO* ANTICANCER ACTIVITY OF *DURANTA ERECTA* L. (VERBENACEAE)

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ABSTRACT: The aim of the present work is the evaluation of anticancer activity of *Duranta erecta* L. stem extracts and also an investigation of the phytoconstituents from the different extracts of the plant. Chloroform, ethyl acetate, methanol and aqueous extracts of *Duranta erecta* L. stem were tested for their anticancer activity on three cancer cell lines, *i.e.*, MCF-7 (Breast), HL-60 (Leukemia), and HT-29 (Colon) at various concentrations. The anticancer effect of the extracts on cell inhibition was studied using Sulphorhodamine B assay. Chloroform, ethyl acetate, and methanolic extracts showed the dose-dependent anticancer effect on leukemia cancer cells, but these extracts were inactive against breast and colon cancer cell lines. The aqueous extract was inactive against all three cell lines. Phytochemical profile of the plant extracts proves the presence of flavonoid, alkaloids, saponins, tannins, and triterpenes. The result suggested that chloroform, ethyl acetate and methanolic extracts of *Duranta erecta* L. contains some important chemical constituents, which can be further used in the treatment of leukemia.

INTRODUCTION: Cancer is one of the most life-threatening diseases and the leading causes of death all over the world. According to cancer statistics in 2013, stomach and liver cancer are the most common in Asia, and both are associated with high mortality rates, while bladder cancer is the most common in the USA. Colorectal and breast cancers have high incidence rates in all countries [1]. According to the estimates of the WHO, more than 80% of people in developing countries depend on traditional medicine for their primary health needs [2].

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The plant kingdom has provided a variety of medicines for cancer treatment, currently, over 60% of the drugs are derived in one or other way from natural source including plant, marine organism and micro-organism [3]. Over the past decade, herbal medicines have been accepted universally, and they have an impact on both world health and international trade. Hence, medicinal plants continue to play an important role in the healthcare system of a large number of the world's population. Traditional medicine is widely used in India. The National Cancer Institute collected about 35,000 plant samples from 20 different countries and has screened around 114,000 extracts for anticancer activity [4].

Treatment by herbal medicines may have some advantages over treatment by single purified chemicals; as herbal medicine are the mixtures of

more therapeutic or preventive components, and so might have more activity than single products alone [5]. The antioxidant and anti-tumor effects of extracts from various herbs and medicinal plants have been proved experimentally and clinically. Several *in-vitro* or *in-vivo* studies have proved the anticancer potential of the extracts from several medicinal plants [6]. A diverse number of anticancer chemical constituents have been isolated from natural sources *viz*. Camptothecin, vincristine, vinblastine, taxol, podophyllotoxin, *etc*. Further modification of these compounds leads to the development of potent anticancer agents like Topotecan, Irinotecan, and etoposide teniposide, *etc*. [7]

Duranta erecta L. is commonly known as pigeon berry belongs to the family Verbenaceae. It is shrubs or herbs usually 1 to 3 m in height [8]. The plant is not browsed by cattle and is believed to be poisonous [9]. Ethyl acetate and aqueous extracts of leaves showed significant antimalarial activity when administered to mice [10]. The fruits are used in the treatment of malaria and intestinal worms [11]. The leaves are used in the treatment of abscess [12]. From the genus *Duranta* several iridoid glycosides as durantosides I, II, III, IV, and lamiide was isolated [13-14]. Flavonoids and C-alkylated flavonoids [15-16] and some alkaloids [17] were isolated.

Based on the literature survey, it is evident that no work has been carried out on the evaluation of the anticancer property of stem extracts on the selected cell lines. Hence in this present study, the anticancer activity of *Duranta erecta* L. stem extracts was assessed by investigating the inhibition of cell growth of MCF-7 (Breast), HL-60 (Leukemia), and HT-29 (Colon) at various concentrations.

MATERIALS AND METHODS:

Plant Materials and Reagents: Stems of *Duranta erecta* L. were collected from Nanded, Maharashtra and authenticated from Botanical Survey of India, Pune. Plant Authentication No. BSI/WRC/100-1/ Tech./2017. All the reagents and chemicals were purchased from Merck Chemicals Ltd.

Preparation of Stem Extract: The stems were washed with distilled water, shade dried and powdered. Powdered drug material was subjected to successive solvent extraction using chloroform, ethyl acetate, methanol, and water respectively. The extracts were filtered, evaporated at 40º and stored at 40º.

Phytochemical Screening: Phytochemical screening was done to determine the presence or absence of secondary metabolites such as tannins, alkaloids, flavonoids, saponins, sterols, and phenolic compounds. This was done according to established procedure [18-21].

Alkaloid Test (Dragendroff's Test): 2 ml plant extract was acidified with few drops of dilute hydrochloric acid. To this acidic medium, 1 ml of Dragendroff's reagent (Potassium bismuth iodide) was added. An orange or reddish brown precipitate produced indicates the presence of alkaloids.

Flavonoid Test (Shinoda Test): The presence of flavonoids was confirmed by treating the alcoholic plant extract with few fragments of magnesium ribbon and hydrochloric acid. The reaction mixture develops pink or crimson red color, indicating the presence of flavonoids.

Saponin Test (Foam Test): 1 ml of each extract shaken with 10 ml of distilled water and it was agitated in a graduated cylinder for 10 min. The formation of persistent honey-comb like froth indicated the presence of saponins.

Carbohydrate Test (Molish's Test): A small amount of extract was treated with an alpha-Naphthol solution in alcohol, shakes and adds conc. H_2SO_4 from the side of the test tubes and observed for the formation of the violet ring at the junction of two liquids.

Tannin Test (Lead Acetate Test): To 2 ml of each extract add a few drops of 10% Lead acetate were added. The appearance of white precipitate indicates the presence of tannins.

Steroids Test: 50% H_2SO_4 is added along the sides of the test tube containing a mixture of methanolic HCl and acetic anhydride. If there is any change in color, from green to blue-green (sometimes *via* red or blue) indicates the presence of terpenoids and steroids.

Phenol Test: When 0.5 ml of $FeCl_3$ (w/v) solution was added to 2 ml of rest solution, the formation of an intense color indicated the presence of phenols.

Protein Test (Biuret Test): To extract add 4% NaOH and a few drops of 1% $CuSO_4$ solution. The appearance of violet or pink color indicates the presence of Protein.

Anticancer Activity: The cell lines were grown in RPMI 1640 medium containing 10% fetal bovine serum and 2 mM L-glutamine. For the present screening experiment, cells were inoculated into 96 well microtiter plates in 100 µL at plating densities as shown in the study details above, depending on the doubling time of individual cell lines. After cell inoculation, the microtiter plates were incubated at 37° C, 5% CO_2, 95% air and 100% relative humidity for 24 h before the addition of experimental drugs.

Different extracts were initially solubilized in dimethyl sulfoxide at 100 mg/ml and diluted to 1mg/ml using water and stored frozen before use. At the time of drug addition, an aliquot of frozen concentrate (1 mg/ml) was thawed and diluted to 100 µg/ml, 200 µg/ml, 400 µg/ml and 800 µg/ml with complete medium containing test article. Aliquots of 10 µl of these different drug dilutions were added to the appropriate microtiter wells already containing 90 µl of the medium, resulting in the required final drug concentrations, *i.e.* 10 µg/ml, 20 µg/ml, 40 µg/ml, 80 µg/ml.

After compound addition, plates were incubated at standard conditions for 48 h and assay was terminated by the addition of cold TCA. Cells were fixed *in-situ* by the gentle addition of 50 µl of cold 30 % (w/v) TCA (final concentration, 10% TCA) and incubated for 60 min at 4 °C. The supernatant was discarded; the plates were washed five times with tap water and air dried. Sulforhodamine B (SRB) solution (50 µl) at 0.4% (w/v) in 1% acetic acid was added to each of the wells, and plates were incubated for 20 min at room temperature. After staining, the unbound dye was recovered, and the residual dye was removed by washing five times with 1% acetic acid. The plates were air dried. The bound stain was subsequently eluted with 10 mM trizma base, and the absorbance was read on a plate reader at a wavelength of 540 nm with 690 nm reference wavelength.

Percent growth was calculated on a plate-by-plate basis for test wells relative to control wells. Percent Growth was expressed as the ratio of average absorbance of the test well to the average absorbance of the control wells * 100 [22-25].

Using the six absorbance measurements [time zero (Tz), control growth (C), and test growth in the presence of drug at the four concentration levels (Ti)], the percentage growth was calculated at each of the drug concentration levels. Percentage growth inhibition was calculated as:

$$[Ti/C] \times 100\% \text{ (Ref)}$$

RESULTS AND DISCUSSION: The result of the preliminary phytochemical analysis is presented in **Table 1**. The phytochemicals present in the stem of *Duranta erecta* L. was found to be alkaloids, saponins, tannins, flavonoid, steroids, and phenolic compounds. The presence of one or more of these secondary metabolites indicated that the anticancer activity is due to these active compounds present in different parts of the plant.

In the present study solvents, extracts of the stem of *Duranta erecta* L. namely chloroform, ethyl acetate, methanol, and aqueous extracts were evaluated for anticancer activity on three cancer cell lines, *i.e.* MCF-7 (Breast), HL-60 (Leukemia), and HT-29 (Colon) at various concentrations. The activities of the different extracts were compared with the standard drug Adriamycin (Doxorubicin). The result was found that chloroform, ethyl acetate, and methanol extracts were active on Human Leukemia Cell Line HL-60 and inactive on Human Breast cancer cell line MCF-7 and Human Colon cancer cell line HT-29. The aqueous extract was inactive against all the three selected cell lines. The GI_{50} value of chloroform, ethyl acetate, methanol and aqueous extracts for MCF-7 and HT-29 cell line was found to be >80 µg/ml and for HL-60 cell line GI_{50} value for chloroform, ethyl acetate, methanol extracts was found to be <10 µg/ml except for aqueous extract >80 µg/ml. Doxorubicin served as a positive control which showed GI_{50} value <10 µg/ml on three cancer cell lines **Table 2, 3** and **4**. The results of *in-vitro* cancer activity showed that *Duranta erecta* plant could be used in the treatment of leukemia and the demonstration of anticancer activity against Human Leukemia Cell Line HL-60 is an indication that there is the possibility of sourcing of new anticancer compounds from screened plant leading to the discovery of new compounds.

TABLE 1: PHYTOCHEMICAL ANALYSIS OF DIFFERENT EXTRACTS OF *DURANTA ERECTA* STEM

Phytochemicals	Different Extracts of *Duranta erecta* stem			
	Chloroform	Ethyl Acetate	Methanol	Aqueous
Alkaloids	+	+	+	-
Phenols	+	+	+	-
Tannins	+	+	+	+
Flavonoids	+	+	+	+
Saponins	+	+	+	+
Steroids	+	+	-	-
Carbohydrates	-	-	-	+
Proteins	-	-	-	+

Where, + = Present, - =Absent

TABLE 2: *IN-VITRO* ANTICANCER ACTIVITY ON MCF -7 CELL LINES

1	% Growth Control				GI_{50}
	Drug concentration µg/ml				
	10	20	40	80	
Chloroform extract	95.9	97.7	86.1	71.3	>80
Ethyl acetate extract	96.2	102.9	94.8	76.5	>80
Methanolic extract	97.5	105.2	101.3	88.1	>80
Aqueous extract	107.2	113.1	114.4	104.7	>80
ADR	-64.3	-64.0	-50.1	-45.9	<10

TABLE 3: *IN-VITRO* ANTICANCER ACTIVITY ON HT-29 CELL LINES

2	% Growth Control				GI_{50}
	Drug concentration µg/ml				
	10	20	40	80	
Chloroform extract	114.8	117.6	119.9	109.4	>80
Ethyl acetate extract	102.0	111.6	112.6	108.7	>80
Methanolic extract	97.1	105.7	102.6	110.3	>80
Aqueous extract	101.1	103.5	110.6	118.7	>80
ADR	-16.1	-22.5	-21.3	-22.4	<10

TABLE 4: *IN-VITRO* ANTICANCER ACTIVITY ON HL-60 CELL LINES

3	% Growth Control				GI_{50}
	Drug concentration µg/ml				
	10	20	40	80	
Chloroform extract	-5.8	-3.5	-10.7	-18.6	<10
Ethyl acetate extract	6.0	27.6	40.3	6.4	<10
Methanolic extract	20.5	37.4	29.5	45.0	<10
Aqueous extract	133.0	91.3	99.3	72.6	>80
ADR	-64.3	-68.7	-72.6	-73.5	<10

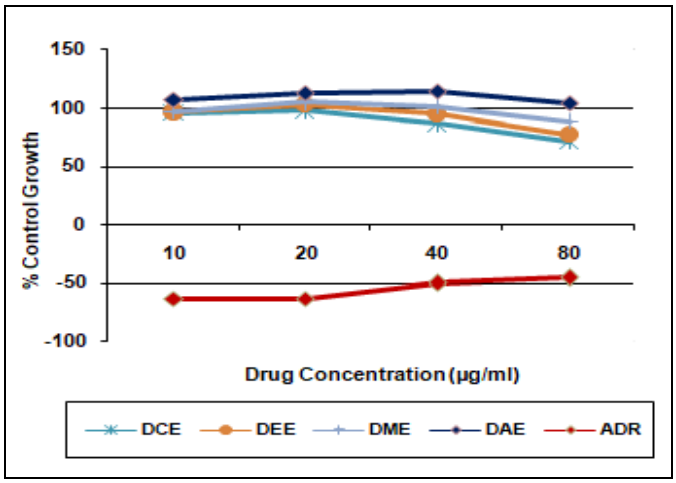

FIG. 1: GROWTH CURVE: HUMAN BREAST CANCER CELL LINE MCF-7

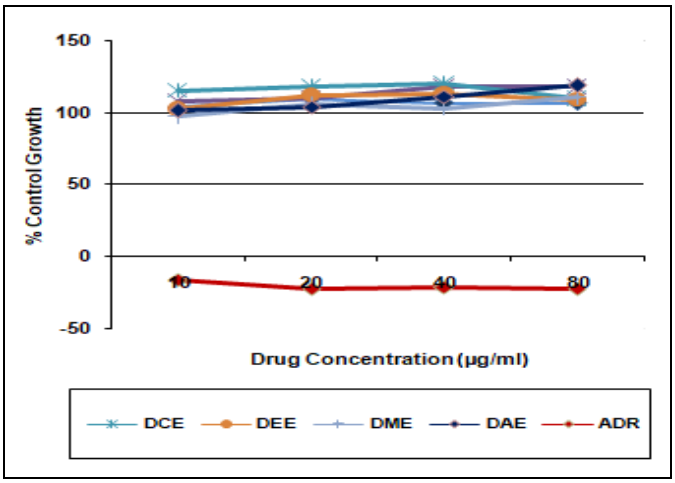

FIG. 2: GROWTH CURVE: HUMAN COLON CANCER CELL LINE HT-29

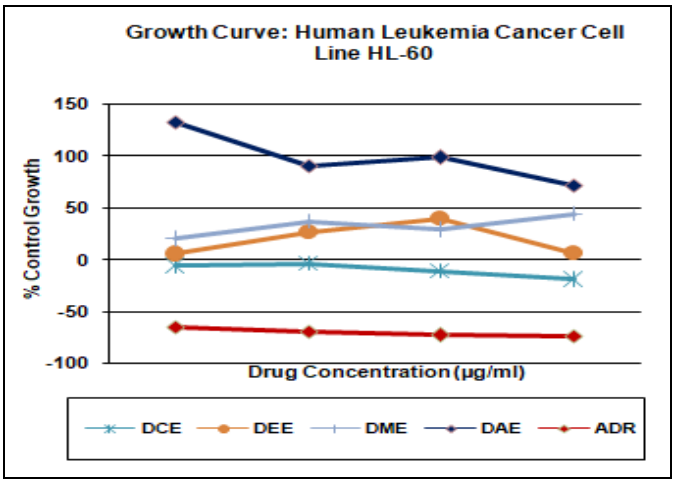

FIG. 3: GROWTH CURVE: HUMAN LEUKEMIA CANCER CELL LINE HL-60

CONCLUSION: In conclusion, *Duranta erecta* stem can be considered as an important source of natural products that have anticancer potential. Further investigation is going on to find out the active compounds responsible for the anticancer activity and their mechanism of action. However, the present study of the *in-vitro* anticancer activity of *Duranta erecta* forms primary Platform for further phytochemical and pharmacological studies.

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CONFLICT OF INTEREST STATEMENT: We declare that we have no conflict of interest.

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