IJPSR (2021), Volume 12, Issue 8 (Research Article)

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 24 January 2020; received in revised form, 21 March 2020; accepted, 08 June 2021; published 01 August 2021

ANTICANCER ACTIVITY OF *EPIPHYLLUM OXYPETALUM* (DC.) LEAVES EXTRACT BY *IN-VIVO IN-VITRO* MODELS

Shraddha Anil Naik [*1] and N. S. Naikwade [2]

Annasaheb Dange College of B. Pharmacy [1], Ashta, Maharashtra, India.
Department of Pharmacology, Appasaheb Birnale College of Pharmacy [2], South Shivaji Nagar, Nishant Colony, Sangli - 416416, Maharashtra, India.

Keywords:

Aberrant crypt foci, N-methylnitrosourea, MTT assay, Trypan blue assay, Histopathology of colon

Correspondence to Author:
Shraddha Anil Naik

Annasaheb Dange College of B. Pharmacy, Ashta - 416301, Maharashtra, India.

E-mail: nshraddha211@gmail.com

ABSTRACT: Colon carcinogenesis is a multistep process that arises by the accretion of genetic alterations. The genetic and epigenetic changes which transform normal colonic epithelium into aberrant crypt foci which is responsible for penetration. The GC-MS analysis of ethanolic extract of *Epiphyllum oxypetalum* (DC.) have shown the presence of phenolic compound (4-hydroxy-2-methylacetophenone), Diterpene, steroids which were reported for antioxidant and anticancer activity. In the present study Brine shrimp lethality, Trypan blue assay, and MTT assay *in-vitro* models are performed. In an *in-vivo* study, animals were divided into 5 groups as Normal, Control (NMU + distilled water 10ml/kg.), Standard (5-Flurouracil with the dose of 10mg/kg via intraperitoneal route given once in a 3 days of interval for 15 days to complete 5 cycles.), Test I (100mg/kg absolute ethanol extract of leaves of plant *Epiphyllum oxypetalum* (DC.)) and Test II (200mg/kg absolute ethanol extract of leaves of plant *Epiphyllum oxypetalum* (DC.)) groups each group comprised 6 animals. Cancer were induced in rats by using N-Methylnitrosourea by intra-rectal instillation method in a dose of 0.4% solution of N-methylnitrosourea 3 times in a week for 3 weeks. After 3 weeks of induction period; Animals in Test I and Test II groups were treated with the dose of 100mg/kg and 200mg/kg of ethanolic extract were administered daily *via* oral for 8 weeks, respectively. It is concluded that *Epiphyllum oxypetalum* (DC.) treatment at dose 200mg/kg showed better anticancer activity when compared to the lower dose.

INTRODUCTION: Colon cancer is the elevated mortality-related significant health hazard that is the second most prevalent cause of cancer-related death. Cancer in India can have deep social and economic implications for individuals. Colorectal cancer is the third leading cause of cancer in both sexes in the United States [2].

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.12(8).4229-39

This article can be accessed online on www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.12(8).4229-39

The typical Western diet is processed meat, animal fats, alcohol, and refined carbohydrates that include elevated protein concentrations are liable for the danger of colorectal cancer. However, there are many food-borne chemicals that play protective roles and help maintain these cells' ideal functional condition [6]. New therapies to treat and deter this life-threatening disease are constantly being demanded. The interest in science and research is drawing their attention to compounds derived from nature that are deemed to have fewer toxic side effects than compared to present medicines such as chemotherapy [3].

The phylloclade of plant *Epiphyllum oxypetalum* (DC.) has certain active components that have shown antibacterial activity. The stem is used for the medicinal treatment of dropsy as well as heart disease. Flower also has the ability to cure the abscesses of the wound. It is also used for cough and bloody phlegm, uterine bleeding, and breath shortness. The chemical content of *Epiphyllum oxypetalum* (DC) leaves has a powerful ability to suppress pain and can neutralize blood coagulation [9].

The preliminary phytochemical study of alcohol extract of leaves *Epiphyllum oxypetalum* (DC.) plant has shown Glycosides existence, Saponins, Steroids, Phenols, Proteins, Resins, Tannins and Terpenoids which were responsible for Probable antioxidant and anticancer activity [10].

MATERIALS AND METHODS:

Cell Line Used: The following cell line were procured from National Cell Culture Science, Pune. (Human colon cell line: COLO 205)

Preparation of Absolute Ethanolic extract of leaves of *Epiphyllum oxypetallum* (DC.) Plant:

Leaves Materials and Pre-treatments: The leaves of *Epiphyllum oxypetalum* (DC.) Haworth plant was collected in the month of July and August 2018 from Bagni, Maharashtra (India). The plant material was authenticated by Prof. M. D. Wadmare, Department of Botany from Smt. Kasturbai Walchand College, Sangli.

Extraction of Leaves and Yield: The powdered leaves material was defeated by using petroleum ether, and again this powder was dried to evapourate petroleum ether, and this powdered material was used for extraction. The extraction was done by using absolute ethanol by using Microwave-assisted extractor method at temperature 75 ^{o}C. The final extract was weighed and the percentage of yield content was calculated using formula:

$$\% \text{ Yield} = \text{Wt. of extract (g)} / \text{Wt. of sample (g)} \times 100$$

Experimental Animals: Wistar rats of either sex aging 7 weeks, were procured from animal house of Appasaheb Birnale College of Pharmacy, Sangli, were used for the study. Form B protocol were prepared and submitted to Institutional Animal Ethics committee (IAEC). Approval for animal use was obtained from IAEC prior to experimental study. The experimental protocol (IAEC/ABCP/06/ 2018-19) was approved by the IAEC.

Housing of the Animals: Animals were housed in well- ventilated room at 23± 2°C, with humidity of 65- 70%, and they were fed with a standard pellet diet with mineral water. Procedures involving laboratory animals were performed in accordance with the guidelines of the Committee for the purpose of Control and Supervision of Experiments on Animals (CPCSEA).

Acute Toxicity Study: Referred to OECD guideline no. 425 to perform acute oral toxicity. Female Wistar rats were procured and kept for overnight fasting before drug administration. *Epiphyllum oxypetalum* (DC.) plant leaves absolute ethanolic extract was administered orally dissolved with distilled water and the dose was calculated based on body weight of Wistar rats. As per the OECD guidelines, three female Wistar rats received a dose of 2000mg/kg. The animals were observed for a time period of 4, 8, 12, and 24 hrs for changes in awareness, mood, CNS activity, muscle tone, reflexes, and autonomic profile. Further, the animals were kept under observation for 15 days for occurrence of any abnormalities [45].

Dose Selection: From the acute toxicity study, the dose of absolute ethanolic leaves extract of the plant *Epiphyllum oxypetalum* (DC.) was safe at the dose 2000mg/kg of body weight. Hence one-20th of 2000 mg/kg, *i.e.*, 100mg/kg body weight, double of thisdose,200 mg/kg body weight of absolute ethanol extract of leaves was chosen to ascertain the response of the animals and also to study the dose-dependency [43].

***In-vitro* Cytotoxicity Screening:**

Brine Shrimp Lethality Assay: Nauplii were collected in a glass pipette along with water, and 10 of such shrimps were transferred to each drug conc. Vial containing 4.5 ml brine solution. In each experiment, 0.5 ml of absolute ethanol extract was added to 4.5 ml of brine solution at various concentrations 20-500µg/ml respectively. In control vial added 4.5 ml of artificial seawater and 0.5ml of distilled water. After 24 h, survival of nauplii were counted by 3X magnifying glass against dark

background, and dose-response data calculated the percentage lethality and LC50 values were transformed into a straight line by means of a trendling [45, 46, 47].

Trypan Blue Dye Cell Exclusion Assay:

Procedure for Cytotoxicity Assay: In the stock cell suspension, cell count was determined, and cells were found COLO 205 cells 2.3×10^5/0.1ml. In the first well added only 0.1ml DMSO (0.1% v/v with PBS-7.4 pH) and considered as a control group. In the next 4 wells, 0.1 ml absolute ethanol extract of concentration ranging from 10, 20, 40 and 100 µg/ml were added in respective micro wells considered as test groups. In the next 4 wells, a concentration of 5-FU were added in respective 4 micro wells of microtiter well plate. Considered as standard groups. Further, microtiter well plate was incubated at temperature 37 ºC and 5% CO_2 incubator for period of 3 h. After the incubation, in each microwell of microtiter well plate individually. 0.1 ml of trypan blue was added and mixed well.

MTT Assay: Seeded 100ul of cell suspension in a 96-well microtiter plate. Incubated the plate at 37°C in a 5% CO_2 atmosphere for the required period of time. After the incubation period, plates were removed from the incubator and added MTT reagent to a final concentration of 10% of total volume. Plate wrapped with aluminium foil and incubated for 2 to 4 h. After incubation period, added 100ul of solubilisation solution to each well.

Gentle stirring on gyratory shaker will enhance dissolution. Occasionally, pipetting up and down may be required to completely dissolve the MTT formazan crystals, especially in dense culture. Read the absorbance on a spectrophotometer or an ELISA reader at 570nm with a reference wavelength of higher than 650nm. Subtract the average 570nm absorbance values of the control wells from the average 570nm absorbance values of corresponding experimental wells. Plotted the absorbance values on the Y-axis and your experimental parameters on the X-axis.

***In-vivo* Anticancer Activity in N-Methyl-nitrosourea induced Colon Cancer in Rats:**

Induction of Colon Cancer in Rats: N-methylnitrosourea.

Induction of Aberrant Crypt Foci: Animals were divided into 5 groups as Normal, Control, Standard, Test I, and Test II groups; each group comprised 6 animals. Cancer were induced in rats by using N-Methylnitrosourea by intra-rectal instillation method in a dose of 0.4% solution of N-methylnitrosourea 3 times in a week for 3 weeks. The solution was prepared immediately before administration, was administered except for the normal group. The intrarectal instillation was done by using a metal feeding tube, and a plastic feeding tube 8 cm long was inserted two-thirds into the colon lumen through the anal orifice, and the solution was instilled.

Treatment Period: After 3 weeks of induction period; Animals in Test I and Test II groups were treated with absolute ethanol extract of leaves of plant *Epiphyllum oxypetalum* (DC.) with the dose of 100mg/kg and 200mg/kg were administered daily *via* oral route to the animals for 8 weeks respectively. The animals in the standard group were treated with 5-Fluorouracil with the dose of 10mg/kg *via* intraperitoneal route given once in a 3 days of interval for 15 days to complete 5 cycles. Control group were administered with only distilled water 10ml/kg. The cancerous animals as well as animals in the normal group, were allowed for free access to tap water and pellet diet and maintained at room temperature in plastic cages. After 8 weeks of treatment, initiation blood was collected by retro-orbital route for estimation of haematological parameters. After 8 weeks, animals were kept on fasting for 24 hours, then sacrificed, and the colon was excised, cut open along its length, rinsed with 0.9% saline solution, and kept in 10% buffered formalin for Histopathological studies. Livers were isolated, homogenized in tris buffer & used for estimation of antioxidant activity.

Evaluating Parameters:

A. Weekly Measurement of Body Weight of Animals: Increase in body weight as compared to day zero body weight.

B. Tumourological Parameters: Morphological views of colon and formation of aberrant crypt foci

C. Haematological Parameters: RBC, WBC, Hb, DLC.

D. Tissue Antioxidant Biomarkers: SOD, GSH, Catalase.

E. Histopathological Study: Histopathological study of colon in all the animals. At the end of study, the intestine were isolated, washed with ice cold tris buffer. Each tissue specimen was fixed in 10% buffered neutral formalin solution. After fixation tissues were embedded in paraffin wax and sections were cut and stained with haematoxylin and eosin. The slides were observed under light microscope 10x & 45X.

Statistical Analysis: Values are expressed as Mean ± SEM for six rats in each group, statistical analysis was performed using one-way ANOVA followed by Dunnett's t test (Graph Pad Instat 7.04, USA). $p<0.05$ was taken as the criterion of statistical significance.

RESULTS:

Brine Shrimp Lethality Bioassay:

TABLE 1: THE MEAN %MORTALITY AFTER 24 H (MEAN ± SEM) ON TREATMENT OF ABSOLUTE ETHANOL EXTRACT OF LEAVES OF *EPIPHYLLUM OXYPETALUM* (DC.) AND 5-FLUOROURACIL ON BRINE SHRIMP LETHALITY BIOASSAY

Treatments	Dilutions µg/ml	Mean % Mortality after 24 h (Mean ± SEM)	LC_{50} (µg/ml)
Standard group:	20	31.00±0.5774	
5-Flurouracil	50	46.44±0.1100	
	100	73.52±0.1867	123.80 µg/ml
	200	82.00 ± 2.517	
	500	93.44 ±0.1100	
Test group: Absolute ethanol extract	20	16.66 ±0.1934	
of leaves of *Epiphyllum oxypetallum*	50	30.33 ±0.3333	
(DC.)	100	53.07 ± 0.3754	241.68 µg/ml
	250	60.33±0.3333	
	500	73.33 ± 0.5774	

TABLE 2: TRYPAN BLUE DYE CELL EXCLUSION ASSAY

Groups	Viable cell count (Mean ± SEM) on COLO205 Cell Line	% viability
Control	87.67±0.6667	79.33±0.3333
Standard (10µg/ml)	33.67±0.5774****	23.24±0.2767
Standard (20µg/ml)	26.67±0.3333****	20.97±0.2467
Standard (40µg/ml)	22.33±0.3333****	14.96±0.03667
Standard (100µg/ml)	12.33±0.3333****	16.88±0.2200
Test I (10µg/ml)	38.00±0.333****	25.53±0.3400
Test II (20µg/ml)	26.67±0.3333****	24.63±0.1667
Test III (40µg/ml)	18.67±0.3333****	23.49±0.4267
Test IV (100µg/ml)	13.33±0.3333****	19.28±0.2168

All the values are expressed Mean ± SEM and n=3, ****P<0.0001 using one way ANOVA coupled with "Dennett's test", criterion for significance. ****P<0.0001 is considered as significant when standard group and test group compared with control group.

MTT Assay:

TABLE 3: *IN-VITRO* CYTOTOXIC EFFECT OF ABSOLUTE ETHANOL EXTRACT OF LEAVES OF *EPIPHYLLUM OXYPETALUM* (DC.) ON COLO205 CELL LINE BY MTT ASSAY METHOD

Treatments	Dilutions µg/ml	% Inhibition	IC_{50} µg/ml
Test group: Absolute ethanol extract of leaves	10	25.54	
of *Epiphyllum oxypetalum* (DC.)	20	28.21	165.61 µg/ml
	40	29.42	
	100	32.19	
Standard group:	10	33.88	
5-Flurouracil	20	34.24	150.14µg/ml
	40	34.24	
	100	35.04	

***In-vivo* Anticancer Activity:** Effect absolute ethanol extract of leaves of *Epiphyllum oxypetalum* (DC.) on % change in body weight during treatment period of 11 week in N-methyl-nitrosourea induced colon cancer

TABLE 4: ANIMAL BODY WEIGHT

Groups with Treatment (n=6)	% change in body Weight from zero-day during a treatment period of 11th week.
Normal D/W (P.O)	↑ 27.3
Control D/W (P.O)	↓ 4.9
Standard 5- FU 10mg/kg (IP)	↑ 15.07
Test I Ab. ethanol extract 100mg/kg (P.O)	↓ 10.33
Test II Ab.ethanol extract 200mg/kg (P.O)	↑ 16.74

Tumourlogical Parameter:

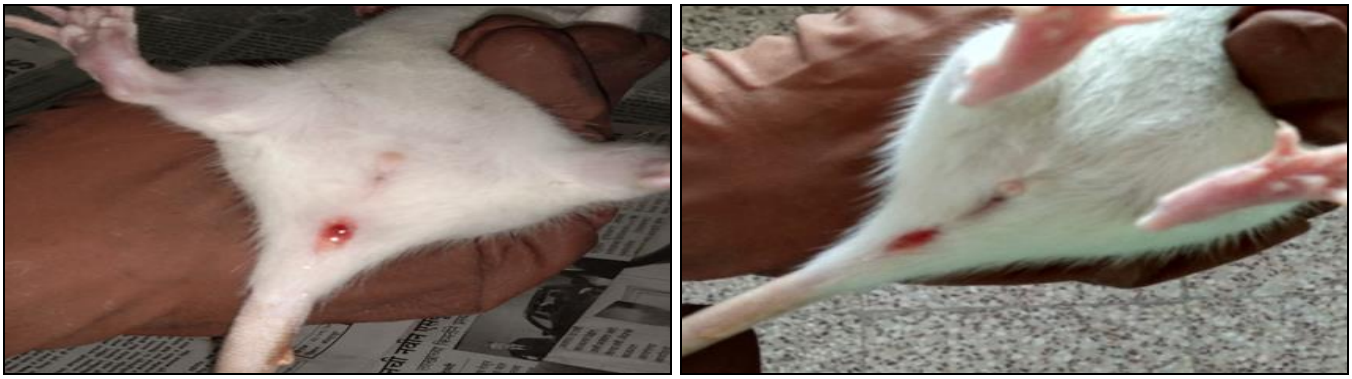


FIG. 1: RECTAL BLEEDING AFTER INDUCTION OF N-METHYL NITROSOUREA: AFTER 2 WEEKS OF INDUCTION PERIOD, ANIMALS SHOWED RECTAL BLEEDING

GROUP I: NORMAL: DISTILLED WATER: THE NORMAL HEALTHY COLON WHICH RECEIVED VEHICLE

GROUP II: CONTROL: EFFECT OF 0.4% SOLUTION OF N-METHYL NITROSOUREA ON COLON TISSUE: THE NUMBER OF ABERRANT CRYPT FOCI WAS OBSERVED IN NMU CONTROL GROUP

GROUP III: STANDARD: EFFECT OF 5-FLUROURACIL ON COLON TISSUE WITH DOSE OF 10mg/kg

GROUP IV: TEST I: EFFECT OF ABSOLUTE ETHANOL EXTRACT OF PLANT LEAVES OF *EPIPHYLLUM OXYPETALUM* (DC.) ON COLON TISSUE WITH DOSE OF 100mg/kg

GROUP V: TEST II: EFFECT OF ABSOLUTE ETHANOL EXTRACT OF PLANT LEAVES OF *EPIPHYLLUM OXYPETALUM* (DC.) ON COLON TISSUE WITH DOSE OF 200mg/kg

FIG. 2: MORPHOLOGICAL VIEWS OF COLON AND FORMATION OF ABERRANT CRYPT FOCI

Hematological Parameters:

TABLE 5: EFFECT OF ABSOLUTE ETHANOL EXTRACT OF LEAVES OF *EPIPHYLLUM OXYPETALUM (DC.)* ON HAEMATOLOGICAL PARAMETERS IN N-METHYL NITROSOUREA INDUCED COLON CANCER IN RATS

Groups n=6	Hb (gm/dl)	RBC (Millions/cu mm)	WBC (Thousands /cumm)	DLC	
				Lymphocytes (%)	Neutrophils (%)
Normal	16.12±0.3439	6.400±0.1789	9808±222.8	72.67±0.9535	18.50±0.3416
Control	12.33±0.2539####	4.250±0.1342####	5100±333.3####	53±0.6325####	11.33±0.4944 ####
Standard	11.37±0.2512*	3.950±0.2975****	5333±200.7	55.83±0.5426	10.50±0.4282
Test I	14.22±0.2512****	5.183±0.01667*	8083±350.2****	65.50±0.8466***	14.50±0.2236****
Test II	14.13±0.1022***	6.133±0.03333****	10083±300.5s****	71.83±0.1667****	16.33±0.3333****

Values are expressed Mean ± SEM and n=6, *P < 0.05, ****P<0.0001 using one way ANOVA coupled with "Dennett's t test", ****P<0.0001 is considered as significant when standard group compared with normal group and #### indicate normal group compared with control group and ***** indicate all other groups (Standard, Test-1 & Test-2) when compared with control.

In RBC count, Test-I group showed 5.183 million cells/cumm, while Test-II group showed 8083 thousand cells/cumm, while Test II group showed significant increase *i.e.* 10083 Thousand/cu mm.

Antioxidant Markers:

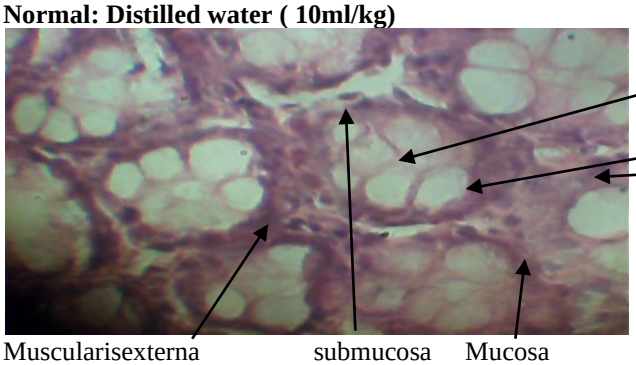
TABLE 6: EFFECT OF ABSOLUTE ETHANOL EXTRACT OF LEAVES OF *EPIPHYLLUM OXYPETALUM (DC.)* ON LIVER TISSUE IN N-METHYL NITROSOUREA INDUCED COLON CANCER IN RATS

Groups (n=6)	Superoxide dismutase (SOD) U/mg of protein	Catalase (CAT) U/mg of protein	Reduced glutathione (GSH) nmol/mg
Normal	38.56±0.8572	22.79±0.4353	13.25±0.05627
Control	11.91±0.3598####	8.633±0.5548####	8.100±0.1483####
Standard	31.29±2.372****	14.48±0.7747****	12.80±0.000****
Test I	19.88±2.372*	11.36±0.7747**	9.400±0.1317****
Test II	14.31±1.903ns	13.83±0.2367****	12.13±0.1054****

Values are expressed Mean ± SEM and n=6, *P < 0.05, ****P<0.0001 using one way ANOVA coupled with "Dennett's t test", ****P<0.0001 is considered as significant. #### indicate control group compared with normal group. **** indicates all other groups (Standard, Test-1 & Test-2) when compared with control and ns indicates non-significant.

Histopathological Study:

Normal: Distilled water (10ml/kg)



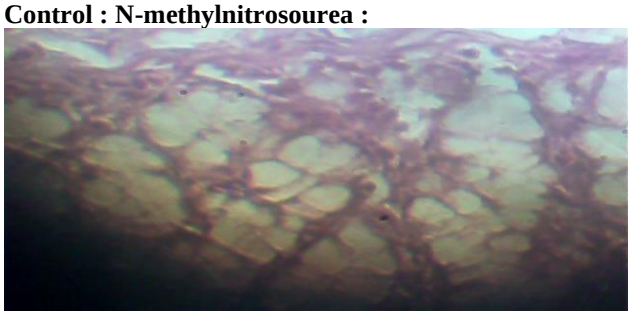
Normal epithelial lining

No necrosis, infiltration of cells.

crypts of Lieberkuhn

A regular arrangement of cells was observed showing normal muscularisexterna, submucosa, mucosa, and crypts of Lieberkuhn.

Control : N-methylnitrosourea :

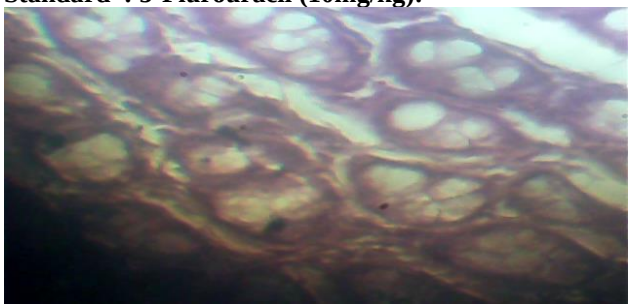


There is a hyperproliferation of cells with submucosa and mucosa being damaged.

Hyperplasia.

Additionally, abscess in crypt of Lieberkuhn, high aberrant crypt foci, and enlargement of nucleus and proliferation of cells have been reported.

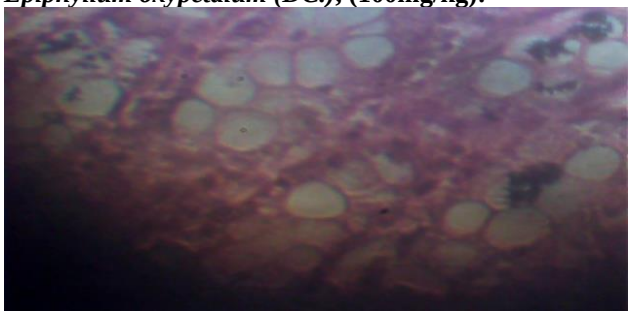
Standard : 5-Flurouracil (10mg/kg):



Less number of hyperproliferative cells and normal structures close to the control group.

Hyperplasia.

Test I: Absolute ethanol extract of leaves of *Epiphyllum oxypetalum (DC.)*, (100mg/kg):

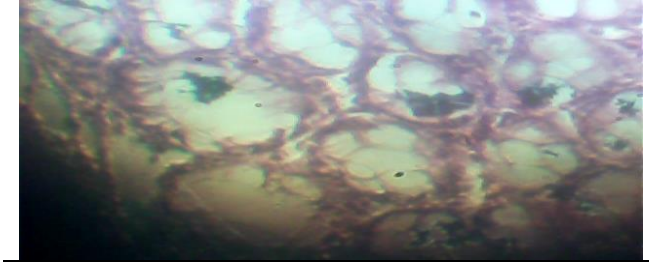


Hyperplasia.

In the Test I group (100mg/kg) Showed less number of hyperproliferative cells, similar to control which is the treatment group at 100mg/kg.

crypts of Lieberkuhn

Test II: Absolute ethanol extract of leaves of *Epiphyllum oxypetalum (DC.)*, (200mg/kg)



crypts of Lieberkuhn

In the Test II group (200mg/kg), as evident in Figure histology of mucosa is near to normal still denoting a few hyperproliferative cells.

Muscularisexterna.

FIG. 3: EFFECT OF 5- FLUOROURACIL AND ABSOLUTE ETHANOL EXTRACT OF LEAVES OF *EPIPHYLLUM OXYPETALUM* (DC.) ON COLON TISSUE IN N-METHYL NITROSOUREA INDUCED COLON CANCER

DISCUSSION: Many plant species are already being used to treat or prevent the development of cancer. The plant *Epiphyllum oxypetalum* (DC.), commonly known as Bramhakamal or queen of Night in India, is an important medicinal plant from the species of the cactus family (Family-Cactaceae) has several traditional uses and forms an important ingredient in Malay traditional medicine. The plant is often cultivated as an ornamental plant used by rural people for medicinal purposes [5]. The Preliminary phytochemical analysis of *Epiphyllum oxypetalum* (DC.) has been reported by Upendra *et al.*, Which showed the presence of Glycosides, Saponins, Steroids, Phenols, Proteins, Resins, Tannins, and Terpenoids. Also in the previous GC-MS analysis of an ethanolic extract of *Epiphyllum oxypetalum* (DC.) has shown the presence of phenolic compound (4-hydroxy-2-methyl aceto-phenone), Diterpene, steroids which were reported for antioxidant and anticancer activity [9]. Antioxidants present in the *Epiphyllum oxypetalum* (DC.) play a crucial role in protecting the cells and tissues against damage caused by reactive oxygen species.

In our study, we observed LC_{50} value of absolute ethanolic extract of the leaves of *Epiphyllum oxypetalum* (DC.) was 241.68 µg/ml in Brine shrimp bioassay, and it would act as cytotoxic agent. According to Clarkson's index, observed LC_{50} value (241.68 µg/ml) classified in medium toxic class, based on the LC_{50} value determined from Brine shrimp lethality bioassay extract that are cytotoxic have good correlation between being effective anticancer agent [46].

The cell viability of the COLO205 cells was assessed by using the trypan blue method. Trypan blue is an energy-dependent dye exclusion viability testing method; the dye is being excluded from live cells. As trypan blue is a weak acid, its affinity is increased for basic proteins; nuclei uptake is generally higher due to the presence of histones, yielding marked blue intensity, whereas the cytoplasm remains faintly stained. This method helped to determine the percentage of viable and dead cells in the absolute ethanolic extract of leaves of *Epiphyllum oxypetalum* (DC.) treated COLO205 cells. The cell line incubated with standard 5-FU and test drug has shown significant cytotoxic effect after 3 hours of incubation; there was a dose-dependent decrease in viable cell count of the treated cells. In different test dilutions, 100µg/ml have shown a minimum number of viable cells *i.e.*, 13.33 when compared to the control group, 87.67. The % viability was 19.28%, when compared with the control group (79.33%).

In this study, the cytotoxic activity of absolute ethanol extract of leaves of *Epiphyllum oxypetalum* (DC.) was also determined by using MTT assay in COLO205 cell line, which was exposed various dilutions *viz*. 10, 20, 40, 100 µg/ml. There was a dose-dependent increase in percentage inhibition, and it was found that at the dose level, 100µg/ml maximum % inhibition was observed *i.e.* 32.19%. While the IC_{50} was found by using linear regression analysis IC_{50} value for the standard was 150.14 µg/ml. and for the test was 165.61 µg/ml.

In the present study anticancer activity was determined by *in-vivo* method; were N-methylnitrosourea induced colon cancer model was chosen, absolute ethanolic extract of *Epiphyllum oxypetalum* (DC.) at dose 100mg/kg(Test-I) and 200mg/kg (Test-II) were screened for anticancer activity. According to the previous studies, NMU induced model, the NMU-bearing rats showed the presence of aberrant crypt foci (ACF). Evidence is supporting the idea that aberrant crypt foci are colon cancer precursors whose size and numbers directly correlate with the risk of developing colon cancer.

Body Weight Changes: Sudden weight loss is often a symptom of several types of cancer, including colon cancer. Additionally, if a tumour in the colon gets large enough, it could block the colon. This blockage can affect bowel habits, which leads to unexplained weight loss [51]. In our study, we have measured weekly changes in of body weight of animals. Initial body weight group was considered as 100%, while all other groups percentage change in body weight was obtained by comparing with Initial body weight in the induction period; Where % increase in body weight from zero days to 3rd week in Normal group showed 6.60% and control group showed 8.87 % reduction in body weight while standard group showed 3.76% reduction in body wt. In contrast, Test I and Test II groups showed 1.13% and 2.76% reduction in body weight respectively. From results, it observed that

the Test-II group showed less reduction in body wt. as compared to Test-I. Percentage change in body weight during a treatment period of 11 weeks were also determined, Initial body weight was considered as 100%, while all other groups percentage change in body weight was obtained by comparing with initial body weight. Where % increase in body weight from zero-day to 11th week in Normal group showed 27.3%. The standard group showed 15.07% increase in body weight. Test I group animals showed a 10.33% reduction in body weight, while Test II animals showed 16.74% increase in body weight after 8 weeks of treatment.

Tumourological Parameters: ACF can be used as a biomarker for disease states, including colon cancer. The most noticeable of all the signs, blood on or in the stool, can be associated with colorectal cancer [28]. After 2 weeks of induction period of NMU, animals showed rectal bleeding. No ACF developed in the Normal group which received vehicle. An increase in the number of ACF was observed in the NMU control group when compared to both Normal and standard group. In the present study, 200 mg/kg absolute ethanol extract of *Epiphyllum oxypetalum* (DC.) showed less number of aberrant crypt foci (ACF).

Hematological Parameters: In order to interpret the effectiveness of the drug, a basic study is necessary to ensure their safety; the different hematological parameters were analyzed. Significant decrease in haemoglobin, RBC may be due to iron deficiency or due to haemolytic or myelopathic conditions [52, 53].

From the interpretation of haematological parameter results by statistical analysis, the RBC count of Test II group was significantly increased 6.133 million/cu mm, when compared to control *i.e.* (4.250 millions/cu mm), while WBC count was 10083/cu mm, showed significant increase when compared with control (*i.e.* 5100 /cu mm). It is also showed improvement in blood cell count as compared to standard.

In differential leucocyte count in Test-II group, both lymphocytes 71.83%, neutrophils 16.33% showed significant increase when compared with control group which have lymphocyte and neutrophils count 53% & 11.33% respectively.

The haemoglobin count of (200mg/kg) extract was significantly increased when compared with control. The haemoglobin count of Test-II was observed (14.13gm/dl), and the count of the control group was observed (12.33gm/dl).

Antioxidant Markers: In the present study, absolute ethanol extract of *Epiphyllum oxypetalum* (DC.) 100mg/kg (19.88 U/mg of protein) and 200mg/kg (14.31 U/mg of protein) treated rats showed a markedly increase in the superoxide dismutase activity in the liver when compared with the control group (11.91 U/mg of protein).

Catalase is a hemoprotein, and it protects cells from the accumulation of H_2O_2 and able to prevent the tissue from reactive free oxygen and hydroxyl radicals, by catalyzing the reduction of H_2O_2 to form H_2O and O_2. The catalase activity in liver tissue homogenate for 200mg/kg (13.83U/mg of protein) and 100mg/kg (11.36U/mg of protein), which showed significant increase when compared with the control group (8.633U/mg of protein). These enzymes serve as a defense system to safeguard the cells from the toxic effect of reactive oxygen intermediates. Reports indicate that many anti-oxidants are anti-carcinogens. In our study, absolute ethanolic extract of leaves of *Epiphyllum oxypetalum* (DC.) showed anti-oxidant properties and may therefore be effective in combating oxidative stress following tumour transplantation.

Reduced glutathione is present in all types of living cells. The intracellular level of this tripeptide varies with the growth, nutritional state, and hormonal balance of the organism. Tissues such as mammalian liver normally contain high levels of reduced glutathione. In present research work, absolute ethanol extract of *Epiphyllum oxypetalum* (DC.) and improve reduced glutathione activity in liver homogenate at dose 200mg/kg (12.13nmol/mg) and 100mg/kg (9.400nmol/mg), when compared with control group (8.25nmol/mg).

Histopathological Study: The extent of damage to the colon by N-methylnitrosourea is evaluated by an increase in ACF count and polyp count. In N-methylnitrosourea control, there are a high number of ACF, adenoma, and polyps. In the 5-Fluorouracil treatment, *Epiphyllum oxypetalum* (DC.) absolute ethanol extract of leaves showed

good anticancer activity by maintaining the low ACF count, polyp count when compared to N-methylnitrosourea control group. Histopathological results of control group showed an abscess in crypt of Lieberkuhn, high aberrant crypt foci, and enlargement of nucleus and proliferation of cells. A lesser number of aberrant crypt foci, hyperplastic cells, and no abscess in mucosal crypts were observed in group treated with 5-fluorouracil and absolute ethanol extract of leaves of *Epiphyllum oxypetalum* (DC.) at dose 100mg/kg and 200mg/kg. In the Test II group (200mg/kg), as evident in histology, the mucosa appears near to normal, still denoting a few hyperproliferative cells compared with the control group.

In summary, the cytotoxic effect of *Epiphyllum oxypetalum* (DC.) absolute ethanol extract of leaves showed concentration-dependent cytotoxic activity to the COLO205 cell line.

The absolute ethanol extract of leaves of *Epiphyllum oxypetalum* (DC.) might be inducing cell death through apoptosis and its mechanism possibly supported by antioxidant assay. Its radical scavenging properties are demonstrated by SOD, CAT, and GSH assay.

Based on our findings, which are comprised of less ACF count, increased antioxidant levels, and restored Histopathological findings, we can conclude that *Epiphyllum oxypetalum* (DC.) treatment at dose 200mg/kg showed better anticancer activity when compared to the lower dose. *Epiphyllum oxypetalum* (DC.) extract can be a promising candidate for treating colon cancer by normalizing levels of antioxidant enzymes, inhibiting hyperplastic cells, decreasing ACF counting to the colon.

The plants containing phenolic compound and their derivatives, Flavones, Terpenoids, and Glycosides *viz. Barleria Prionitis* (L.) were reported for promising anticancer activity. The presence of these constituents probably responsible for antioxidant and anticancer activity [15].

CONCLUSION: In the present study, anticancer activity was determined by the *in-vivo* method; were N-methyl nitrosourea induced colon cancer model was chosen, absolute ethanolic extract of *Epiphyllum oxypetalum* (DC.) at dose 100mg/kg (Test-I) and 200mg/kg (Test-II) were screened for anticancer activity. According to the previous studies, NMU induced model; the NMU-bearing rats showed the presence of aberrant crypt foci (ACF). Evidence is supporting the idea that aberrant crypt foci are colon cancer precursors whose size and numbers directly correlate with the risk of developing colon cancer. In the present study, animals were administered with NMU by intrarectal instillation and observed percentage body weight changes, antioxidant status, tumourological parameter, and histology of colon.

ACKNOWLEDGEMENT: The authors acknowledge Dr. S. A Tamboli, Principal Appasaheb Birnale College of Pharmacy, Sangli, for providing the necessary facilities and infrastructure to perform this study.

CONFLICTS OF INTEREST: Nil

REFERENCES:

1. Mallath MK, Taylor DG, Badwe RA, Rath GK, Shanta V, Pramesh CS, Digumarti R, Sebastian P, Borthakur BB, Kalwar A and Kapoor S: The growing burden of cancer in India: epidemiology and social context. The Lancet Oncology 2014; 205-12.
2. Murthy NS, Chaudhry K and Rath GK: Burden of cancer and projections for 2016, Indian scenario: gaps in the availability of radiotherapy treatment facilities. Asian Pac J Cancer Prev 2008; 671-7.
3. Greenwell M and Rahman PK: Medicinal plants: their use in anticancer treatment. International Journal of Pharmaceutical Sciences and Research 2015; 4103.
4. Ali R, Mirza Z, Ashraf GM, Kamal MA, Ansari SA, Damanhouri GA, Abuzenadah AM, Chaudhary AG and Sheikh IA: New anticancer agents: recent developments in tumor therapy. Anticancer Research 2012; 2999-3005.
5. Upendra RS and Khandelwal P: Assessment of nutritive values, phytochemical constituents and biotherapeutic potentials of *Epiphyllum oxypetalum*. International Journal of Pharmaceutical Sciences 2012; 421-5.
6. Swerdlow AJ: Second cancer risk after chemotherapy for Hodgkin's lymphoma: a collaborative British cohort study. Journal of Clinical Oncology 2011; 4096-104.
7. Sisodiya PS: Plant derived anticancer agents: a review. Int J Res Dev Pharm L Sci 2013; 293-308.
8. Kaur R, Kapoor K and Kaur H: Plants as a source of anticancer agents. J Nat Prod Plant Resour 2011; 119-24.
9. Dandekar R, Fegade B and Bhaskar VH: GC-MS analysis of phytoconstituents in alcohol extract of Epiphyllum oxypetalum leaves, Journal of Pharmacognosy and Phytochemistry 2015; 148-54.
10. Mahmad A, Shaharun MS, Saad B and Dash GK: *Epiphyllum oxypetalum* Haw.: a lesser known medicinal plant. Indo American Journal of pharmaceutical Sciences 2017; 10.
11. Sirhi B: Indian Council of Medical Research consensus document for the management of colorectal cancer. Indian Journal of Medical and Paediatric Oncology: Official Journal of Indian Society of Medical & Paediatric Oncology 2014; 192.
12. Asaduzzaman M, Rana MS, Hasan SM, Hossain MM and Das N: Cytotoxic (brine shrimp lethality bioassay) and antioxidant

investigation of *Barringtonia acutangula* (L.). Int J of Pharm Sci and Res 2015; 1179-85.
13. Perse M and Cerar A: The Dimethylhydrazine induced colorectal tumours in rat-experimental colorectal carcinogenesis. Radiology and Oncology 2005; 1-39.
14. Shukla S and Gunjegaokar SM: Pharmacognostical and pharmacological profiling of *Barleria prionitis* Linn. Journal of Biological Sciences and Medicine 2018; 4(1): 41-50.
15. Gupta RS, Singh K and Sharma D: comprehensive review on *Barleria Prionitis* (L.). Asian journal of Pharmaceutical and Clinical Research 2017; 22-29.
16. Salunkhe NB, Kadam AP, Aparadh VT and Chavan JJ: Comparative study of photosynthetic pigments and phenolic content in three Barleria species. World J Pharm Res 2013; 626-30.
17. Nema R, Khare S, Jain P, Pradhan A, Gupta A and Singh D: Natural products potential and scope for modern cancer research. American Journal of Plant Sciences 2013; 1270.
18. Avelar-Freitas BA: Trypan blue exclusion assay by flow cytometry. Brazilian Journal of Medical and Biological Research 2014; 307-15.
19. Tripathi KD: Essentials of Medical Pharmacology. Jaypee Brothers Medical Publishers (P) Ltd. 7th ed. 2013; 857-870.
20. Chari KY, Polu PR and Shenoy RR: An Appraisal of Pumpkin Seed Extract in 1, 2-Dimethylhydrazine Induced Colon Cancer In Wistar Rats, Journal of Toxicology, Vol 2018; 1-12
21. Johnson RL and Fleet JC: Animal models of colorectal cancer. Cancer and Metastasis Reviews, 2013; 39-61.
22. Hejmadi M: Introduction to cancer biology. Bookboon; vol 2009, 1-46.
23. Hamilton SR:Vogelstein LH *et al*.: Tumours of the Colon and Rectum, 104-147
24. Mohan H: Textbook of Pathology, Jaypee Brothers Medical Publishers (P) Ltd 5th ed, vol 2008; 550-660.
25. Wargovich MJ, Brown VR and Morris J: Aberrant crypt foci: the case for inclusion as a biomarker for colon cancer. Cancers, Vol 2010 Sep; 1705-16.
26. Arends MJ: Pathways of colorectal carcinogenesis. Applied Immunohistochemistry & Molecular Morphology, Vol 2013; 97-102.
27. Song M, Garrett WS and Chan AT: Nutrients, foods, and colorectal cancer prevention. Gastroent 2015; 1244-60.
28. Danaei G, Vander Hoorn S, Lopez AD, Murray CJ and Ezzati M: Comparative Risk Assessment collaborating group Cancers. Causes of Cancer In The World: comparative risk assessment of nine behavioural and environmental risk factors. The Lancet 2005; 1784-93.
29. Smith RA, Cokkinides V, von Eschenbach AC, Levin B, Cohen C, Runowicz CD, Sener S, Saslow D and Eyre HJ: American Cancer Society guidelines for the early detection of cancer. CA: A Cancer Journal for Clinicians 2002; 8-22.
30. Avelar-Freitas BA, Almeida VG, Pinto MC, Mourao FA, Massensini AR, Martins-Filho OA, Rocha-Vieira E and Brito-Melo GE: Trypan blue exclusion assay by flow cytometry. Brazilian J of Medical and Biological Research 2014; 307-15.
31. The National Toxicology Program (NTP) Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM). Test Method Protocol for the NHK Neutral Red Uptake Cytotoxicity Assay Phase III Validation Study 2003; 1-3.
32. Feoktistova M, Geserick P and Leverkus M: Crystal violet assay for determining viability of cultured cells. Cold Spring Harbor Protocols 2016; 4: 873-79.
33. Hawley TS and Hawley RG: Flow cytometry protocols. Methods in molecular biology. 2nd ed. Humana press, Springer Science & Business Media 2004; 125-30.
34. Borra RC, Lotufo MA, Gagioti SM, Barros FD and Andrade PM: A simple method to measure cell viability in proliferation and cytotoxicity assays. Brazilian Oral Research 2009; 255-62.
35. Voigt W: Sulforhodamine B assay and chemosensitivity. Methods in molecular Medicine 2005; 39-48.
36. Shailah A, Siti A, Noor AM., Suzana M, Wan Z and Yasmin A: Ginger extract (*Zingiber officinale*) triggers apoptosis and G0/G1 cells arrest in HCT 116 and HT 29 colon cancer cell lines. African Journal of Biochemistry Research 2010; 134-42.
37. Meyer M, Essack M, Kanyanda S and Rees JG: A low-cost flow cytometric assay for the detection and quantification of apoptosis using an anionic halogenated fluorescein dye, Biotechniques 2008; 317-20.
38. Rosenberg DW, Giardina C and Tanaka T: Mouse models for the study of colon carcinogenesis. Carcinogenesis 2008; 183-96.
39. Thirupathi K, Krishna DR, Kumar BR, Tirumala RP and Mohan GK: Anticonvulsant Activity of Pericarpium Extract of *Balanites Roxburghii* Planch in Mice. Pharmacol 2009; 1150-57.
40. Khandelwal KR: Practical Pharmacognosy Technique and Experiments, 21st ed, Pune, Nirali Prakashan 2011; 25-29.
41. Meyer BN, Ferrigni NR, Putnam JE, Jacobsen LB, Nichols DJ and McLaughlin JL. Brine shrimp: a convenient general bioassay for active plant constituents. Planta Medica 1982; 31-4.
42. Hamed El-Batanony N: Antimicrobial activities and mode of action of the selected novel thienopyrimidines derivatives 2-[2-(diphenylmethylene) hydrazino]-5-isopropyl-3-methylthieno [2, 3-d] pyrimidin-4-one. Periodicum Biologorum 2017; 27-36.
43. Kini S and Swain SP: Synthesis and evaluation of novel benzothiazole derivatives against human cervical cancer cell lines. Indian Journal of Pharmaceutical Sciences. 2007; 69(1): 46-7.
44. Narisawa T and Fukaura Y: Inhibition by Chlorella of N-methylnitrosourea-induced aberrant crypt foci in rat colon. Food factors for Cancer prevention, Springer, Tokyo, 1997; 577-80.
45. Narisawa T and Fukaura Y: N-methylnitrosourea-induced colon tumorigenesis by ursodeoxycholic acid in F344 rats. Japanese Journal of Cancer Research. 1998; 1009-13.
46. George PA, Tynga IM and Abrahamse H: *In-vitro* antiproliferative effect of the acetone extract of *Rubus fairholmianus* gard Root on Human Colorectal Cancer Cells, BioMed Research International 2015; 1-40.
47. Harzallah HJ: Thymoquinone: The *Nigella sativa* bioactive compound, prevents circulatory oxidative stress caused by 1, 2-dimethylhydrazine in erythrocyte during colon postinitiation carcinogenesis. Oxidative Medicine and Cellular Longevity 2012; 18: 1-6.
48. Saleem TH: Possible protective effects of quercetin and sodium gluconate against colon cancer induction by dimethylhydrazine in mice. Asian Pac J Cancer Prev 2015; 5823-8.
49. Heikkila RE, Cabbat FS and Cohen G: *In-vivo* inhibition of superoxide dismutase in mice by diethyl-dithiocarbamate. J Biol Chem 1976; 251(7): 2182-85.

How to cite this article:
Shraddha AN and Naikwade NS: Anticancer activity of *Epiphyllum oxypetalum* (DC.) leaves extract by *in-vivo in-vitro* models. Int J Pharm Sci & Res 2021; 12(8): 4229-39. doi: 10.13040/IJPSR.0975-8232.12(8).4229-39.

All © 2013 are reserved by the International Journal of Pharmaceutical Sciences and Research. This Journal licensed under a Creative Commons Attribution-NonCommercial-ShareAlike 3.0 Unported License.

This article can be downloaded to **Android OS** based mobile. Scan QR Code using Code/Bar Scanner from your mobile. (Scanners are available on Google Playstore)