ISSN: 2582-6433

INTERNATIONAL JOURNAL FOR LEGAL RESEARCH AND ANALYSIS

Open Access, Refereed JournalMulti Disciplinary
Peer Reviewed6th Edition

VOLUME 2 ISSUE 7

www.ijlra.com

 ISSN: 2582-6433

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ISSN: 2582-6433

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ABOUT US

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Phytochemical Screening And Anticancer Activity Of Cordia Myxa Extract On MCF-7 Cell Line

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Abstract:

Background: Breast cancer is a malignant tumor (a collection of cancer cells) arising from the cells of the breast. Although breast cancer predominantly occurs in women, it can also affect men. While breast cancer rates are higher among women in more developed regions, rates are increasing in nearly every region globally. Cordia myxa (CA) widely known as Lasura consist of flavonoids, triterpenes, tannins, alkaloids and saponins etc which are founded effective in many studies for diseases as well as in cancer. The present study on the malignant using cordia myxa extract has shown significant inhibition of cancer cell of breast cancer with IC 50 of 222 on MCF-7 cell line. On the other hand Doxorubicin which is an anticancer drug shown very promising inhibition of MCF-7 cell line at IC 50 of 23.68.

Keywords: Breast carcinoma, MCF-7 cell line, Cordia myxa.

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1. Introduction

Cancer is a collective term for hordes of diseases characterized by invasion of neighbouring parts of the body and / or pathological cells that can spread to other organs, reproducing beyond their normal limits. Other common terms are malignant tumors and neoplasms. Cancer affects almost all parts of the body, and there are many anatomical and molecular subtypes that require specific management strategies. Cancer is the second leading cause of death in the world, and an estimated 9.6 million people will die by cancer in 2018. Prostate cancer, lung cancer, stomach cancer, liver cancer, colorectal cancer are the most common cancer among men, cervical cancer, lung cancer, colorectal cancer, breast cancer, thyroid cancer are the most common among women (Bray F et al., 2018) .

Breast cancer is a malignant tumor (a collection of cancer cells) arising from the cells of the breast. Although breast cancer predominantly occurs in women, it can also affect men (Larsen MJ et al.,2014). Breast cancer is the most frequent cancer among women, impacting 2.1 million women each year, and also causes the greatest number of cancer-related deaths among women. In 2018, breast cancer is the 2nd most frequent cancer with an estimated that 627,000 women died around the world from breast cancer – that is approximately 15% of all cancer deaths among women. While breast cancer rates are higher among women in more developed regions, rates are increasing in nearly every region globally (Bray F et al.,2018) . Breast cancer can invasive to other body organ and cause the lungs, liver, colon, rectal, vaginal and cervix cancer (Bargagna P et al.,2007). Inflammatory Breast Cancer (IBC) and triple-negative breast cancer are the invasive type of breast cancer while ductal carcinoma and lobular carcinoma are the non-invasive type of breast cancer (Sun H et al.,2017). Early sign and symptoms of breast cancer are typically not recognizable, but the most common symptom is a lump or mass in the breast or underarm area, nipple discharge or redness, changes in the breast skin texture such as puckering or dimpling (like an orange skin), swelling of part of the breast (Hoadley KA et al., 2018).

Many therapies are used such as surgery, radiation therapy, biological therapy (targeted drug therapy), hormone therapy and chemotherapy for breast cancer but these are having more serious side effects as well as very costly. All these therapy have many serious side effects that's why we are going on herbal formulation. At present time natural treatments are used globally at

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higher rate as compare to allopathic medicines (Bargagna-Mohan P et al.,2007). Herbal treatments have similar action to chemotherapy and radiation therapy. Combinational formulations of herbals plants/oils are an attractive option to treat the cervical cancer and breast cancer due to less side effects, less cost and gives the synergistic effects for treating the cervical cancer and breast cancer. It might become a satisfactory remedy for prevention and management of cervical cancer and breast cancer (Shi YQ et al.,2009).

Cordia myxa (family- Boraginaceae), about 300 species have been identified worldwide, mostly in warmer regions . The tree keeps its leaves for most of the year. These are broad, alternate, ovateelliptic shaped. The inflorescence carries numerous white flowers. Fruits are round to ovoid shaped drupes, about 15–20 mm in diameter, arranged in clusters. Their white-yellow color turns blackish when dry. The pulp, very tough and mucilaginous, is edible and has a sweetish flavor (K. Thirupathi et al.,2008) . The plant parts like fruits, leaves, stem bark, seeds and roots of most species of plants of the genus Cordia, especially Cordia dichotoma, C. myxa,C. oblique, C. verbenacea, C. martinicensis, C. salicifolia, C. spinescens, C. latifolia, C. ulmifolia, among others, has long been used in traditional medicine for cicatrizant, astringent, antiinflammatory, anthelminthic, antimalarial, diuretic, febrifuge, appetite suppressant, cough suppressant and to treat urinary infections, lung diseases and leprosy (Enas R et al.,2019*)* . The pharmacological studies carried out with extracts and purified compounds indicates that the plants of Cordia species possess analgesic, anti-inflammatory, antimicrobial, antiparasitic, insecticidal, cardiovascular, respiratory, gastrointestinal and protective effects. Various compounds like flavonoids, triterpenes, tannins, alkaloids and fatty acids possessing wide range of bioactivities were isolated from different plant parts of Cordia species (Ali Esmail Al-Snafi 2016). The fruit has been widely used to treat respiratory infections, coughing, and sore throat and used as a diuretic. The pulp is used to mature abscesses, calm rheumatic pain, treat ringworm, and as an anthelminthic (Tarik Shwaish and Faris JM Al-Imarah2017).

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Flowers Stem bark Ripened fruit

Experimental

2.1. Material and method

2.1.1 Collection of plant materials and sample preparation

The *cordia myxa leaves* were collected from herbal garden of Bank colony, Bhiwani (Haryana). The plant was taxonomically identified by Dr. S.S Yadav, Assistant professor, Department of Botany, Maharshi Dayanand University, Rohtak (Haryana). The whole plant was washed with water and were chopped in small pieces, dried at room temp, grinded in powder form.

2.1.2 Extraction procedure

The powdered sample was extracted using Soxhlet apparatus.Hydroalcohol (50% ethanol and 50% distilled water) solvent 50ml/g of sample weight was measured and filled in to the apparatus. The time of extraction was 18 h for each sample.A total of 50 g of dried powder was packed in the timble of soxhlet apparatus and was extracted using hydroalcohol refluxing at 50-70°C which yielded a dark brown extract. The stock extract was putted in desiccator

2.1.3 Phytochemical screening of the extract by Qualitative test

Sr. No	Test	Name of test	Ethanol extract of cordia myxa
1	Alkaloids	Dragendorff's test	+ve
2	Glycosides	Brontragar,s	+ve
3	Terpenoids	Noller test	-ve
4	Carbohydrates	Fehling test	+ve

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5	Proteins	Ninhydrine test	+ve
6	Steroids	Salkowski reaction	+ve
7	Flavonoids	Shinoda test	+ve
8	Phenols	Lead acetate test	+ve
9	Saponins	Frothing test	-ve

1. Alkaloids: Extract was treated with few drops Dragendroff's reagent(solution of potassium bismuth iodide) orange coloured precipitate indicates a positive result.

2. Glycosides: the extract boiled with dilute sulphuric acid, filtered and to the filtrate benzene,or ether or chloroform is added and shaken well. The organic layer is separated to which ammonia is added slowly.the ammoniacal layer shows pink to red colour indicates the positive result.

3.Terpenoids : the substance was warmed with tin and thionyl chloride . did not show pink colour indicates negative results.

4. Carbohydrates: These two solutions should be stoppered and stored until needed. Mix 15 ml of solution-A with 15 ml of solution -B add 2 ml of this mixture to an empty test tube.Add 3 drops of the Extract to be tested to the tube. Place the tube in a water bath at 60 c. Green suspension and red precipitate indicates a positive result.
5. Proteins: The extracts were treated with a few drops of Nin-hydrin reagent show yellow colour precipitate indicates a positive result.

6.Steriods: extract treated with Salkowski reagent to show the pinkish or dark red colour indicates the positive result.

7. Flavonoids: A few drops of concentrated hydro chloride acid were added to the extract; the formation of dark red color indicates the positive result.

8.Phenols : 2 ml of test solution and 2 ml of Sodium hydroxide and boil for a minute. And add a drop of lead acetate solution. A Brown or a black precipitate indicates a positive result.

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9. Saponins: Extract was mixed with water and shaken; formation of froth that's stable for 15 minutes . and then 1 ml extract was treated with 1 percent lead acetate solution . formation of white colured precipitate not formed indicates negative result.

Materials & Method

1.MTT Powder (the solution is filtered through a 0.2 μ m filter and stored at 2–8 °C for frequent use or frozen for extended periods)

2. DMSO (Dimethyl sulfoxide) can significantly inhibit cancer cell invasion, migration, proliferation and colony formation capabilities through upregulation of HLJ1 in a concentration-dependent manner . DMSO led to a significant increase in the percentage of UV- induced apoptotic cells.DMSO to arrest the cell cycle of cell lines at the G1phase.

3. CO2 incubator : CO2 incubator is used to culture cells to provide it with the optimum temperature, moisture (sterile environment) to maintain optimum pH.

4. Tecan Plate reader: Easy to use multimode plate reader affordable, measure absorbance, high performance detection solutions.

2.2.1. Preparation of test solutions

For cytotoxicity studies, 32mg/ml stocks were prepared using DMSO and serial two fold dilutions were prepared from 320μg/ml to 10μg/ml using DMEM plain media for treatment.

2.2.2 Cell lines and culture medium

All the cell lines were procured from ATCC, stock cells was cultured in DMEM supplemented with 10% inactivated Fetal Bovine Serum (FBS), penicillin (100 IU/ml), streptomycin (100 μg/ml) in a humidified atmosphere of 5% CO_2 at 37^{o}C until confluent. The cell was dissociated with cell dissociating solution (0.2 % trypsin, 0.02 % EDTA, 0.05 % glucose in PBS). The viability of the cells are checked and centrifuged. Further, 50,000 cells /well was seeded in a 96 well plate and incubated for 24 hrs at 37^{o}C, 5 % CO_2 incubator.

2.2.3 Anticancer Evaluation Assay

The monolayer cell culture was trypsinized and the cell count was adjusted to 1.0 x 105 cells/ml using respective media containing 10 % FBS. To each well of the 96 well microtiter plate, 100 μl of the diluted cell suspension (50,000 cells/well) was added. After 24 h,

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when a partial monolayer was formed, the supernatant was flicked off, washed the monolayer once with medium and 100 µl of different test concentrations of test drugs were added on to the partial monolayer in microtiter plates. The plates were then incubated at 37^{o}C for 24hrs in 5% CO_2 atmosphere. After incubation the test solutions in the wells were discarded and 100 µl of MTT (6 mg/10 ml of MTT in PBS) was added to each well. The plates were incubated for 4 h at 37^{o}C in 5% CO_2 atmosphere. The supernatant was removed and 100 µl of DMSO was added and the plates were gently shaken to solubilize the formed formazan. The absorbance was measured using a microplate reader at a wavelength of 590 nm. The percentage growth inhibition was calculated using the following formula and concentration of test drug needed to inhibit cell growth by 50% (IC_{50}) values is generated from the dose-response curves for each cell line.

2.2.4. Calculating Inhibition:

% Inhibition = 100 – (OD of sample/OD of Control) x 100.

2.2.5 Result and discussion

IC_{50} Value

The half maximal inhibitory concentration (IC_{50}) is a measure of the effectiveness of a compound in inhibiting biological or biochemical function. This quantitative measure indicates how much of a particular drug or other substance (inhibitor) is needed to inhibit a given biological process (or component of a process, i.e. an enzyme, cell, cell receptor or microorganism) by half.

The IC_{50} of a drug can be determined by constructing a dose-response curve and examining the effect of different concentrations of antagonist on reversing agonist activity. IC_{50} values can be calculated for a given antagonist by determining the concentration needed to inhibit half of the maximum biological response of the agonist. IC_{50} values for cytotoxicity tests were derived from a nonlinear regression analysis (curve fit) based on sigmoid dose response curve (variable) and computed using Graph Pad Prism 6 (Graph pad, SanDiego, CA, USA)

Nonlinear regression

In statistics, nonlinear regression is a form of regression analysis in which observational data are modeled by a function which is a nonlinear combination of the model parameters and depends on one or more independent variables. The data are fitted by a method of successive approximations.

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Table 1. Anticancer screening results of extracted compound

MCF-7	**Standard**			
mpound Name	**Conc. µM**	**OD @ 590nm**	**% Inhibition**	**IC_{50} in µM**
Control	0	0.828	0.00	
Doxorubicin	3.13	0.602	27.31	
	6.25	0.554	33.12	
	12.5	0.438	47.10	
	25	0.335	59.53	**23.68**
	50	0.253	69.40	
	100	0.135	83.72	

The inhibition level increase with concentration

Table 2. Anticancer screening results of extracted compound

MCF-7	**Cordia myxa extract**			
mpound Name	**Conc. µg/ml**	**OD @ 590nm**	**% Inhibition**	**IC_{50} µg/mL**
Control	0	0.828	0.00	
Sample	25	0.693	16.28	
	50	0.602	27.26	
	100	0.510	38.41	**222**
	200	0.435	47.49	
	400	0.286	65.50	
	800	0.186	77.50	

Result increase with concentration

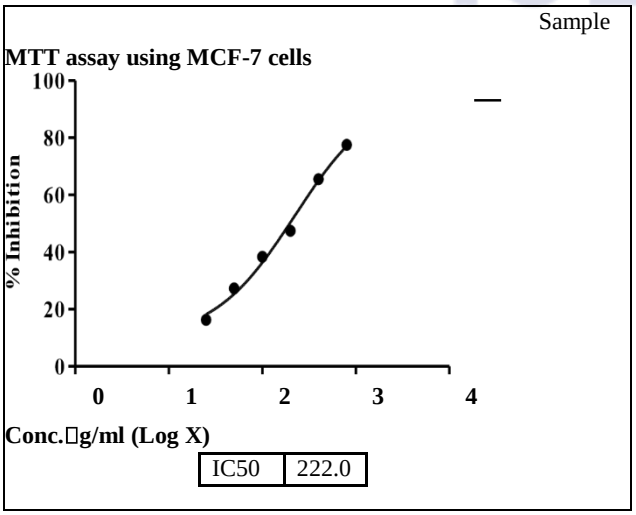

Fig 1

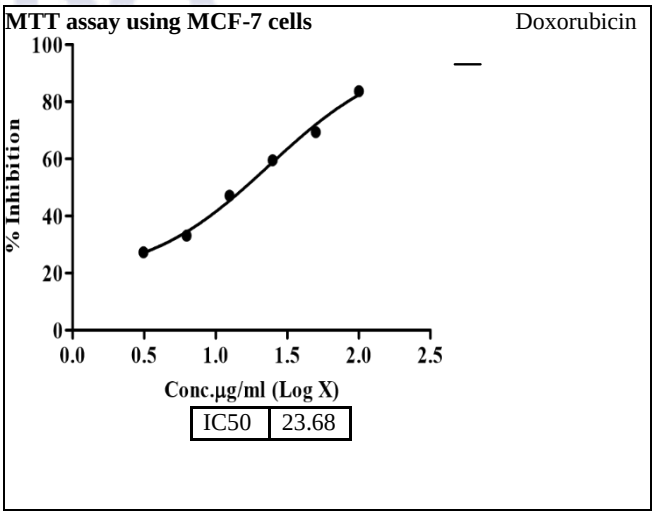

Fig 2

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Conclusion

In present the extract has shown IC50 value of 222µg/mL of inhibition in MCF-7 cells. Standard Doxorubicin has showed 23.68µM inhibition in the same cell line.

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