

In vitro study on *Acalypha wilkesiana* and *Dalbergia sissoo* (Roots) against HT-29 colon cancer cell line

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ABSTRACT

Ethnopharmacological relevance: *Acalypha wilkesiana* used in African folklore for treating cancer were investigated. The traditional uses of *Dalbergia sissoo* bark and leaves uses as anticancer activity. The literature survey found that *Acalypha wilkesiana* and *Dalbergia sissoo* plants can provide anticancer activity and the root part of the plants did not work

Aim: There is an increasing interest among researchers in searching for new anticancer drugs from natural resources, particularly plants. This study aimed to investigate the anticancer properties of *Acalypha wilkesiana* and *Dalbergia sissoo* roots extract against the HT-29 colon cancer cell line.

Materials and methods: The present study has been made to study the anticancer activity on the roots part of medicinal plant *Acalypha wilkesiana* and *Dalbergia sissoo* using pet ether, chloroform, ethyl acetate, ethanol and water extract. The dried part of plant materials of *Acalypha wilkesiana* and *Dalbergia sissoo* were subjected for Hot Continuous successive Extraction (Soxhlet) by using different solvents according to increasing polarity (Petroleum ether, Chloroform, Ethyl acetate, Ethanol, and water). The anticancer activity of *Acalypha wilkesiana* and *Dalbergia sissoo* roots extract on proliferation of HT-29 cancer cell line was determined by 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide (MTT) microculture tetrazolium viability assay. Cytotoxicity assay was carried out using HT-29 colon cancer cell line. The Colon cancer cell was exposed to different concentration 10,1,0.1,0.01, and 0.001. the better solvent extract is determined by the IC₅₀ value.

Results: MTT assay was carried out to observe the despotic effects of various extracts of *Acalypha wilkesiana* and *Dalbergia sissoo* on the growth of HT-29 Human colon cancer cell line and the results are represented in (Table 1 and 2) Five completely diverse concentrations (10,1,0.1,0.01,0.001 µg/mL) of pet ether, chloroform, ethyl acetate, ethanol and water roots extract of *Acalypha wilkesiana* and *Dalbergia sissoo* were applied. The in vitro cytotoxicity against breast cancer cell lines showed potential cytotoxic activity. The results obtained are showed in (table 1 and 2) The results clearly indicate the concentration dependent inhibition of HT-29 cells, with an IC₅₀ value. The IC₅₀ value gives sigmoidal curve so logarithmic method is applied.

Conclusions: The Pet.ether extract, Chloroform extract, Ethyl acetate extract, Ethanol extract and Water extract of *Acalypha wilkesiana* and *Dalbergia sissoo* of roots had demonstrated cytotoxic activity against HT29 cell lines. According IC₅₀ value among the extracts, the ethanolic extract showed better activity than the other solvent extract.

Keywords: *Acalypha wilkesiana*, *Dalbergia sissoo*, MTT assay, HT-29 cancer cell line, IC₅₀ value

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1 Introduction

Cancer is a broad term for diseases in which abnormal cells divide without control and can invade nearby tissues. It can affect any part of the body and is one of the leading causes of death worldwide. The growth of cancer cells forms tumors (except in cases like leukemia, where abnormal cells are present in the blood and bone marrow), and these tumors can spread to other parts of the body, a process called metastasis (Hanahan 2011, Kola 2022). The World Health Organization (WHO) reports that 10 million cancer deaths occurred in 2020, and 18 million new cases were diagnosed globally. By 2040, the incidence is projected to rise to 30 million new cases annually (Bray 2021).

Cancer staging is a crucial part of cancer diagnosis and treatment planning. Staging describes the extent of cancer in the body, including the size of the primary tumor, whether it has spread to nearby lymph nodes, and whether it has metastasized to distant parts of the body. The stage of cancer plays a key role in determining prognosis and selecting the appropriate treatment (Berthelsen 2023). Carcinomas are the most common type of cancer and originate in epithelial cells, which line the inside and outside of the body. These cancers are typically found in the skin, lungs, breast, colon, and other organs (Bray 2018). Colon cancer, also known as colorectal cancer (CRC), is one of the leading causes of cancer-related morbidity and mortality worldwide. It primarily originates in the large intestine (colon) or rectum, with a significant number of cases starting from polyps benign growths that can develop into malignant tumors over time. The pathogenesis of colon cancer

involves a complex interplay of genetic, environmental, and lifestyle factors. (Siegel 2020). Colon cancer has several stages: 0, I, II, III, and IV. Treatment for stages 0 to III typically involves surgery, while for stage IV and the recurrent colon cancer both surgery and chemotherapy are the options (PDQ cancer 2002). The identification of natural compounds in herbal medicines that can be used as anti-cancer drugs has significantly reduced the mortality of cancer patients. In addition, natural compounds derived from medicinal plants have excellent anticancer effects as well as unique advantages such as low toxicity, fewer side effects, and lower cost. Several studies have focused on the therapeutic effects of herbal medicines on metastatic cancers. For example, cancer treatment using berberine, curcumin, resveratrol, and other bioactive compounds extracted from medicinal plants has been shown to target different factors that regulate tumor growth and metastasis. In contrast, the malignancy of cancer cells is typically caused by a combination of cellular and molecular factors (Aime 2024, Ark 2021, Yin2013). Extracts of medicinal plants are useful in the treatment of several health problems such as bacterial (Solanki et al., 2010), including diabetes, cancer and infectious diseases. Antidiabetic, antioxidant, antiarrhythmic, hepatoprotective, antiulcer, immunomodulator, CNS depressant, ant hysteria, antipsychotic, ant gonorrheal infection (Bharti et al., 2011), antiviral, as well as hypolipidemic antifertility etc (Auddy et al., 2003). Reported the aerial part of *Dalbergia sissoo* are anticancer activity (Yasmeen et.al 2021). *Acalypha wilkesiana* used in African folklore for treating cancer were investigated (Osayemwenre

et.al.2014). The traditional uses of *Dalbergia sissoo* bark and leaves uses as anticancer activity (Bloda et.al.2016). The literature survey found that *Acalypha wilkesiana* and *Dalbergia sissoo* plants can provide anticancer activity and the root part of the plants did not work.

2 Material and method:

Collection of Plant Material

Plant used for the study is *Acalypha wilkesiana* and *Dalbergia sissoo*, collected from the Toranmal hill station, Nandurbar district, Maharashtra, India. The plant materials were authenticated by authorized person from i) Botany Department of PSGVPM' Arts, Science, Commerce College, Shahada ii) Botanical survey of India, Pune.

Preparation of plant extracts:

The collected roots of both plants are taken for primary processing i.e. washing to get rid of sand or soil material adhered to the roots. The washing was done by using tap water twice. The cleaned big or long roots are cut into small pieces size 2c.m. to 5c.m. (size reduction) The reduced size material of both plants was taken for shade drying technique. The dried roots of both plants are separately taken from pulverization by using grinding to get coarse powder. The dried part of plant material/s of *Acalypha wilkesiana* and *Dalbergia sissoo* were subjected for Hot Continuous successive Extraction (Soxhlet) by using different solvents according to increasing polarity. (Petroleum ether, Chloroform, Water, Ethyl acetate, Ethanol).

Cytotoxic assay: MTT [(3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide)] is a pale-yellow substrate that is cleaved by living cells to yield a dark blue formazan product. This process requires active mitochondria, and even freshly dead cells do not cleave significant amount of MTT. Thus, the amount of MTT cleaved is directly proportional to the number of viable cells present, which is quantified by colorimetric methods. This assay was performed at Deshpande Laboratories, Bhopal using the standard operating procedures. Briefly the compounds were dissolved in DMSO and serially diluted with complete medium to get the concentrations a range of test concentration. DMSO concentration was kept < 0.1% in all the samples MCF-7 cells maintained in appropriate conditions were seeded in 96 well plates and treated with different concentrations of the test samples and incubated at 37 °C, 5% CO₂ for 72 hours. MTT reagent was added to the wells and incubated for 4 hours; the dark blue formazan product formed by the cells was dissolved in DMSO under a safety cabinet and read at 550nm. Percentage inhibitions were calculated and plotted with the concentrations used to calculate the IC₅₀ values.

3 Results

3.1 Effect of *Acalypha wilkesiana* roots extract of various solvents

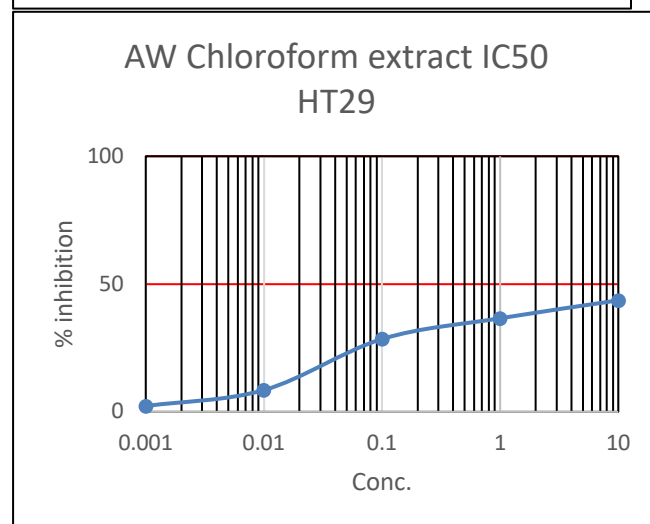
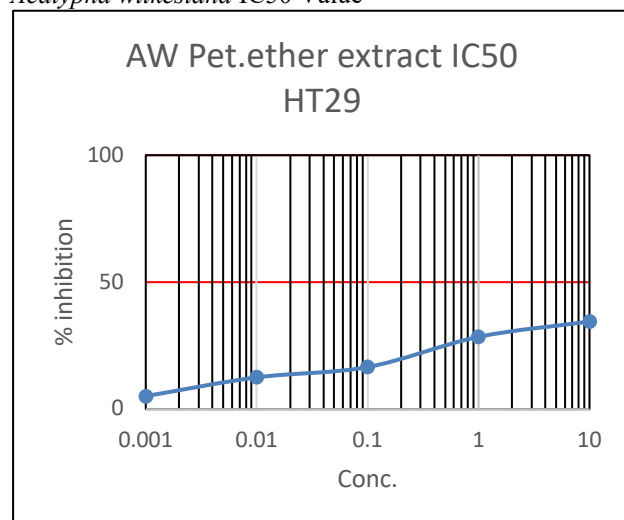
In this assessment, we have the tendency to utilize the MTT test to assess the cytotoxic impact of the pet ether, chloroform, ethyl acetate, ethanol and water roots extract of *Acalypha wilkesiana* against on HT-29 Human Colon cancer cell line.

Table:1 IC₅₀ value of *Acalypha wilkesiana* roots extract of various solvents

Concentration	AW Pet ether	AW Chloroform	AW Ethyl acetate	AW Ethanol	AW Water
10	34.51 ± 0.03	43.62 ± 0.01	52.24 ± 0.04	57.51 ± 0.04	51.27 ± 0.17

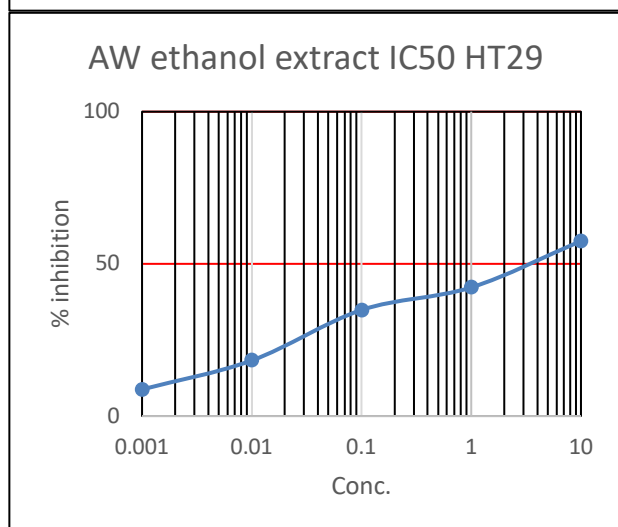
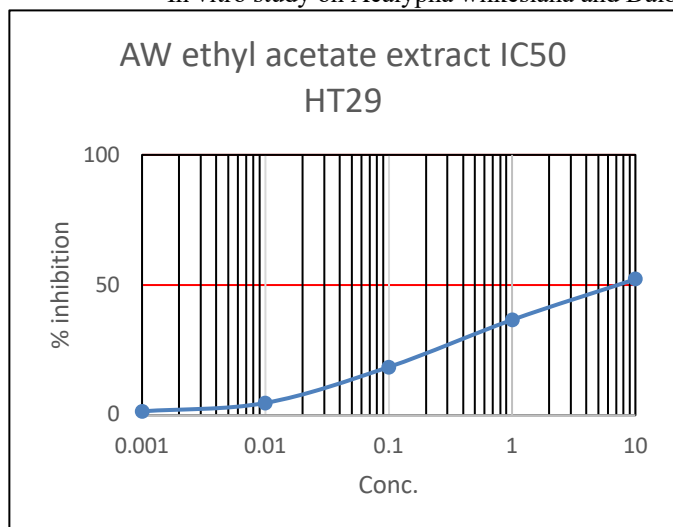
1	28.46 ± 0.04	36.61 ± 0.01	36.62 ± 0.04	42.26 ± 0.05	37.71 ± 0.01
0.1	16.59 ± 0.08	28.48 ± 0.07	18.52 ± 0.02	34.84 ± 0.04	18.52 ± 0.05
0.01	12.52 ± 0.07	8.51 ± 0.01	4.74 ± 0.03	18.26 ± 0.04	5.62 ± 0.02
0.001	5.14 ± 0.03	2.26 ± 0.04	1.15 ± 0.04	8.74 ± 0.03	1.75 ± 0.04
IC ₅₀ Value µg/ml	>10	>10	10	3	7
DOXO IC ₅₀	0.1	0.2	0.2	0.1	0.1

Graphical Representation of Various roots extracts of *Acalypha wilkesiana* IC₅₀ Value



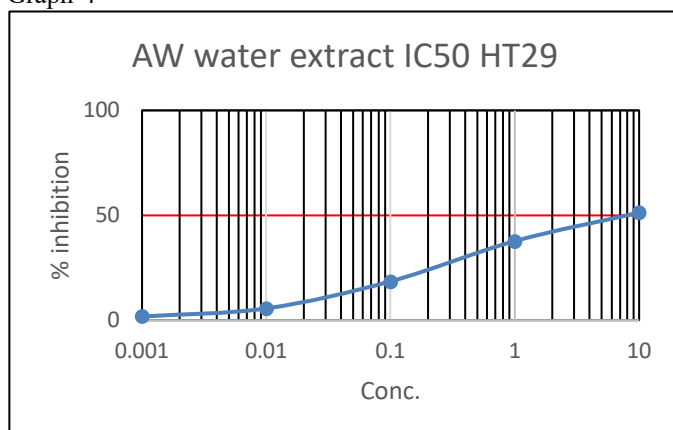
Graph-1

Graph-2



Graph-3

Graph-4



Graph-5

3.1 Effect of *Dalbergia sissoo* roots extracts of various solvents

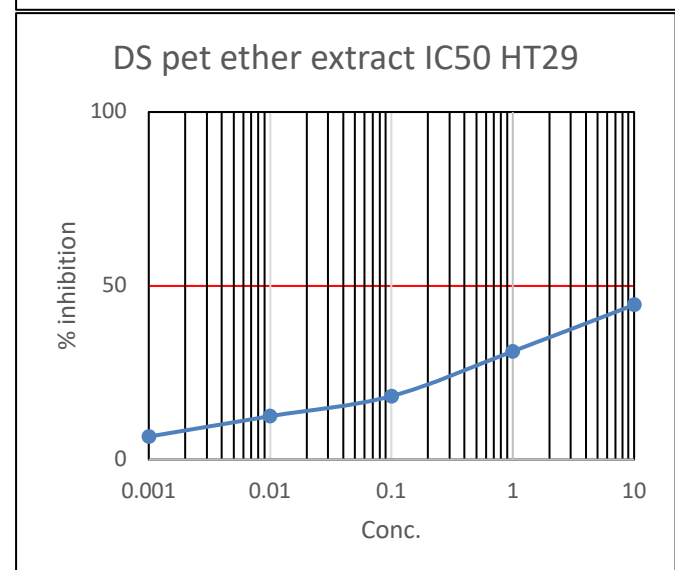
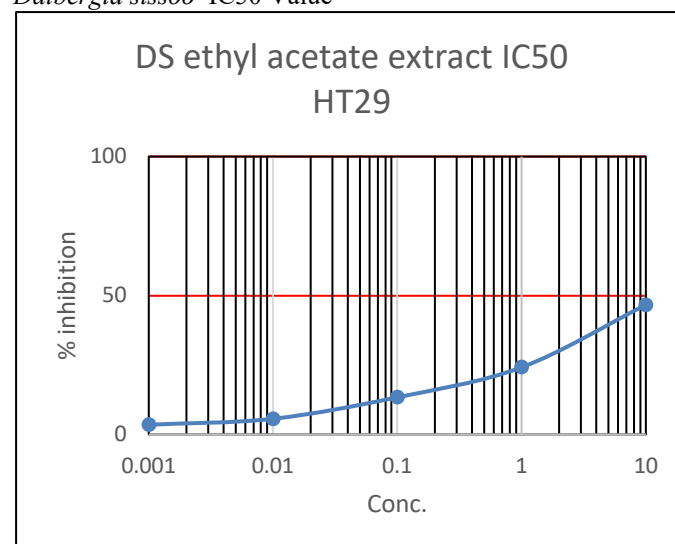
MTT test to assess the cytotoxic impact of the pet ether, chloroform, ethyl acetate, ethanol and water roots extract of *Dalbergia sissoo* against on HT-29 Human Colon cancer cell line.

Table:2 IC50 value of *Dalbergia sissoo* roots extract of various solvents

Concentration	DS Pet ether	DS Chloroform	DS Ethyl acetate	DS Ethanol	DS Water
10	44.5 1 ± 0.11	38.24 ± 0.01	46.62 ± 0.01	54.15 ± 0.04	51.2 7 ± 0.05
1	31.1 5 ± 0.04	28.11 ± 0.10	24.15 ± 0.02	43.62 ± 0.01	35.5 1 ± 0.06
0.1	18.2 6 ± 0.04	16.25 ± 0.02	13.35 ± 0.24	18.59 ± 0.20	12.2 9 ± 0.09
0.01	12.4 8 ± 0.06	14.14 ± 0.05	5.62 ± 0.01	12.51 ± 0.06	4.51 ± 0.10
0.001	6.61 ± 0.06	8.55 ± 0.03	3.47 ± 0.04	8.84 ± 0.01	2.68 ± 0.06
IC50 Value µg/ml	>10	6	>10	2	>10
DOXO IC50	0.1	0.2	0.2	0.1	0.1

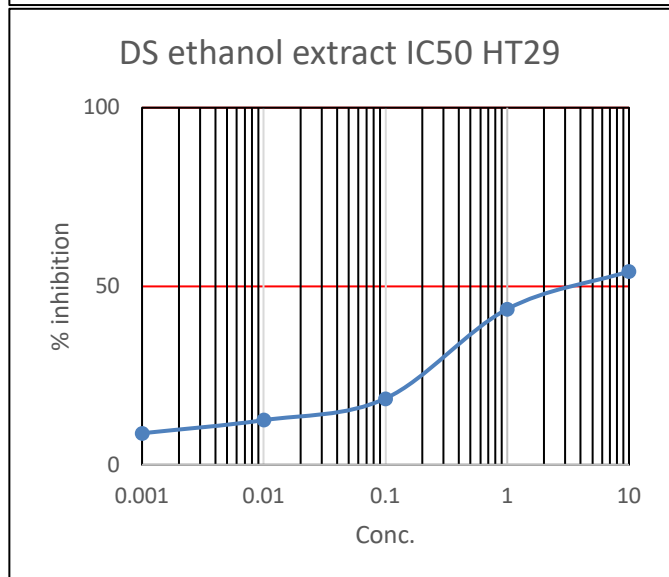
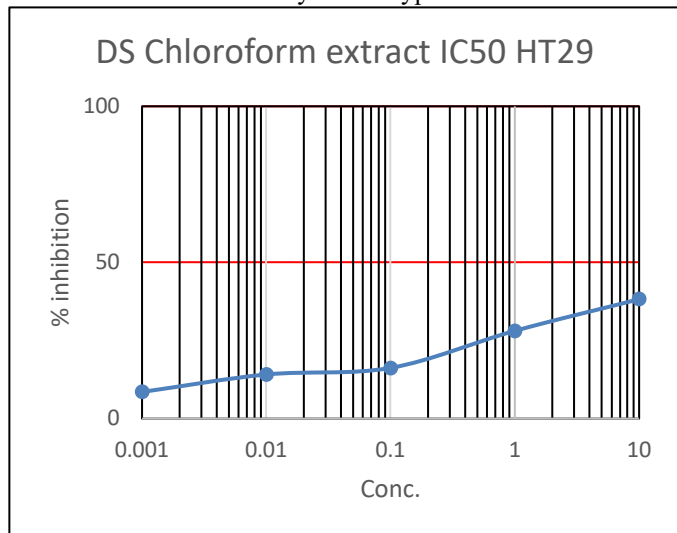
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0.001	6.61 ± 0.06	8.55 ± 0.03	3.47 ± 0.04	8.84 ± 0.01	2.68 ± 0.06
IC50 Value µg/ml	>10	6	>10	2	>10
DOXO IC50	0.1	0.2	0.2	0.1	0.1

Graphical Representation of Various roots extracts of *Dalbergia sissoo* IC50 Value



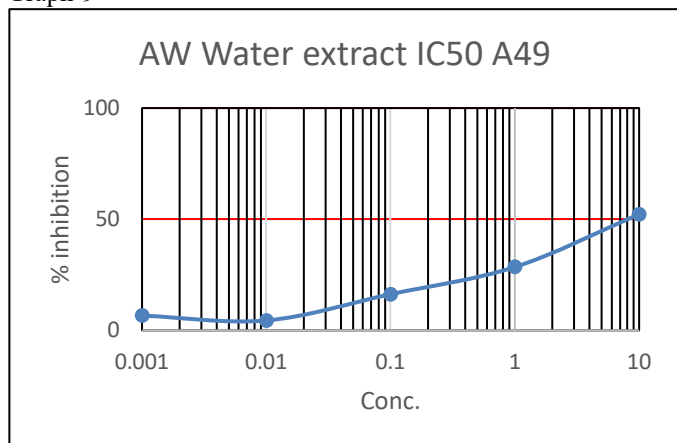
Graph-6

Graph-7



Graph-8

Graph-9



Graph-1

MTT assay was carried out to observe the despotic effects of various extracts of *Acalypha wilkesiana* and *Dalbergia sissoo* on the growth of HT-29 Human colon cancer cell line and the results are represented in (Table 1 and 2) Five completely diverse concentrations (10,1,0.1,0.01,0.001 µg/mL) of pet ether, chloroform, ethyl acetate, ethanol and water roots extract of *Acalypha wilkesiana* and *Dalbergia sissoo* were applied and compre with std drug Doxorubicin.

The in vitro cytotoxicity against breast cancer cell lines showed potential cytotoxic activity. The results obtained are showed in (table 1 and 2) The results clearly indicate the concentration

dependent inhibition of HT-29 cells, with an IC 50 value. The IC50 value gives sigmoidal curve so logarithmic method is applied.

CONCLUSION

The Pet.ether extract, Chloroform extract, Ethyl acetate extract, Ethanol extract and Water extract of *Acalypha wilkesiana* and *Dalbergia sissoo* of roots had demonstrated cytotoxic activity against HT29 cell lines. According IC50 value among the extracts, the ethanolic extract of Both plants showed better activity than the other solvent extract.

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