International Research Journal on Advanced Engineering and Management
https://goldncloudpublications.com
https://doi.org/10.47392/IRJAEM.2025.0488
e ISSN: 2584-2854
Volume: 03
Issue: 10 October 2025
Page No: 3078 - 3083

Anti-Inflammatory and Anti-Cancer Activity of Shoot Tip Exudate of Gardenia Resinifera Roth

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Abstract
Gardenia resinifera Roth (Rubiaceae), different parts of this plant like leaf, fruit and bark are detailed to be a part of traditional medicine system as a cure for extensive ailments. In plant-based isolation and standardisation of bioactive compounds, plenty of plant samples were required. Shoot tip exudate can be collected without harming plant but require time and patience. The pungent smell and taste of the shoot tip is an indication for volatile phytochemicals in the exudate. Hence this study rivets on the bioactive potential of Gardenia resinifera shoot tip exudate especially invitro anti-inflammatory and anticancer studies. The result of the study shows the implicit possibility of shoot tip exudate for the identification of bioactive compounds with anti-inflammatory and anticancer properties. The shoot tip exudate indicated its conquest efficiency of curbing COX and LOX enzyme in inflammation development. MTT assay uncovered the anticancer property of shoot tip exudate which were comparable with standard. Gas Chromatography with Mass Spectral analysis revealed the presence of five compounds which were already reported to have different bioactive properties. This study clearly explains the hidden possibility of bioactive efficacy of Gardenia resinifera shoot tip exudate.
Keywords: *Anti-inflammatory, anticancer, shoot tip exudate, cytotoxicity, GC-MS analysis*

1. Introduction
Plants are natural factories which synthesis potential bioactive compounds and are conducive for humans for their sustainable development. Medicinal plants and plant-based formulations enhanced the quality of human life in all aspects from food to medicines (Yu et al. 2021). Plats have been exploited and screened widely for the identification of bioactive compounds. Progressive analytical technologies paved way for large scale screening of phytochemicals and their bioactive properties (Patel et al. 2021). As part of natural defense mechanism tissues develop inflammation and this inflammation management failure can lead to the oncogenic transformation of cells and tissues. Therefore, inflammation and tumor developments are always analyzed together for proper screening of phytochemicals which can prevent un wanted cell proliferation.

The common name for Gardenia resinifera Roth is Dikamali belong to family Rubiaceae (Hindole et al. (2018). Stem bark, fruit and leaves of Gardenia resinifera has wide range of application in traditional and modern medicine systems (Lakshmi and Jaganmohan reddy 2011, Vindhya and Leelavathi 2015). Resins usually oozes out from bark or shoot tip of plants depending on the characteristics of plant. Phytochemical screening of different species of Gardenia unveiled its importance for further study and use in drug development (Murthy 2010). In the present study the resinous exudate from shoot tip was used for anti-inflammatory and anti-cancer studies.

2. Material And Methods
The shoot tip exudate of Gardenia resinifera was collected and powdered, which was dissolved in suitable solvents for further analysis [1-3]

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2.1.In vitro Anti-inflammatory Assay

RAW 264.7 cell lines (NCCS, Pune, maintained in DMEM media with 10% FBS, Himedia, India) were activated with 1 µL lipopolysaccharide (LPS) (1 µg/mL) and they were exposed to different concentration of test sample and standard. After 24 hours of incubation the anti-inflammatory assays like cyclooxygenase activity and lipoxygenase activity were measured using the cell lysate (Axelrod et al. 1981, Walker and Gierse 2010, Shaikh et al.2016).

2.2.Anti-cancer or Cytotoxicity assessment (MTT Assay)

Mouse breast cancer cell line (C127I) (NCCS, Pune, maintained in DMEM media, supplemented with 10% FBS, 100 µg /mL penicillin and 100 µg/mL streptomycin and kept at 37ºC in an incubator with 5% CO_2) was used for the study. Cytotoxicity of the test materials was performed by MTT (3-(4,5-Dimethylthiazol-2-yl)- 2,5-Diphenyltetrazolium Bromide) assay (Mosmann 1983). Approximately $1x10^5$ cells/mL were seeded in a 24 well plate, with complete growth medium (DMEM) and allowed to attach and grow. At 80% confluency, the medium was replaced with fresh medium containing different concentrations of samples (0-100 µg/mL) and incubated for 48 hrs. At the end of incubation period, the medium was again replaced with fresh medium. Then 40 µL of 5mg/ mL MTT were added to each well and incubated for 4 hrs. The formazan crystals formed were dissolved in dimethyl sulfoxide and the absorbance was measured at 570 nm in ELISA microplate reader (BioTek, USA). The percentage viability was calculated using the formula. [16]

$$\% \text{ viability } = \frac{\text{Absorbance of sample}}{\text{Ababsorbance of control}} X\ 100$$

2.3.GC -MS analysis

The Gas Chromatographic analysis was conducted for the identification of volatile compounds in the sample. The specifications of the column used are-ELITE-5MS column 30 M length, 0.25 mm ID, 0.25 µm thickness, the pressure is 65.0 Kpa and injection temperature was set to 260.00° c. The column oven temperature is 80.0°c. Total flow is 24.0mL/min. and column flow are 1.0 mL/min.1 µL sample was injected in a slit mode. The interpretation on GC-MS was carried out using the database of NIST 11& WILEY 8. [4-6]

3. Results And Discussion

Inflammation is a protective response that is initiated either after injury, physical and chemical damage, or infection by microorganisms, but persistent inflammation may cause chronic diseases. Inflammation is caused by the body release of hormone-like substances called prostaglandins (PGs) and leukotrienes (LTs). These inflammation inducing agents are produced from arachidonic acid (AA) by the enzymes cyclooxygenase (COX) and lipoxygenase (LOX). COX is the first enzyme in the pathway for producing PG and thromboxane (Tx) from arachidonic acid (Jacob et al. 2018). Different species of Gardenia and different plant parts like leaves and fruits were analysed for its anti-inflammatory properties (Koo et al. 2006, Ansari et al. 2019, Chen et al. 2021). The shoot tip exudate of Gardenia resinifera showed cyclooxygenase (COX) inhibitory activity and Lipoxygenase (LOX) inhibitory activity. The shoot tip exudate of Gardenia resinifera inhibited cox enzyme activity by 31.07% at 100mg/ml which is comparable with a standard drug which shoed percentage inhibition of 52.98. The LOX enzyme inhibitory property was found to be same as that of COX in which the shoot tip exudate and standard showed percentage inhibition of 34.75 and 64.83 respectively at a concentration of 100mg/ml (Table 1, Figure 1&2, Figure 3). This is an indication that the test sample has the potential to be developed as non-steroidal anti-inflammatory drugs (NSAIDs). [7-10]

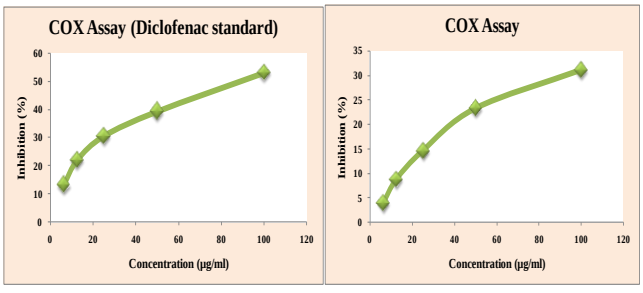

Figure 1 Graph Showing the Percentage Inhibition of COX Enzyme Activity by the Standard and Sample

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Table 1 The percentage Inhibition of COX and LOX Enzyme Activity By Diclofenac Standard And Test Sample (Gardenia Resinifera Shoot Tip Exudate)

Anti-inflammatory Property		COX enzyme activity				
	Concentration	6.25 mg/ml	12.5 mg/ml	25 mg/ml	50 mg/ml	100 mg/ml
Standard	Percentage inhibition	13.10	21.93	30.49	39.23	52.98
Test sample	Percentage inhibition	3.86	8.74	14.59	23.25	31.07
		LOX enzyme activity				
Standard	Percentage inhibition	10.65	17.68	31.54	55.32	64.83
Test sample	Percentage inhibition	4.07	8.67	18.93	28.71	34.75

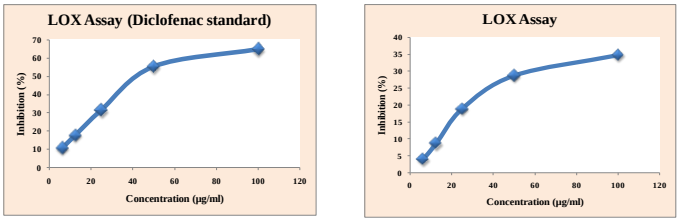

Figure 2 Graph Showing The Percentage Inhibition Of LOX Enzyme Activity By The Standard And Sample

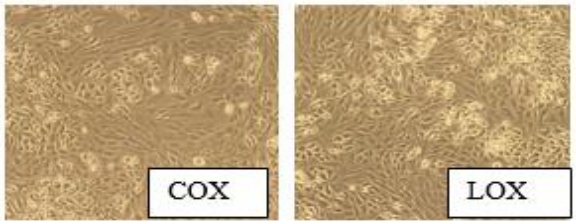

Figure 3 RAW Cells Showing Reduced Inflammatory Activity In The Presence Of Gardenia Resinifera Shoot Tip Exudate During COX Assay and LOX Assay (10X Magnification Under Inverted Phase Contrast Microscope)

Screening of cytotoxicity effect of shoot tip exudate of Gardenia resinifera was performed by MTT assay. It is revealed from the results that the treated cell lines are affected by different concentrations of extract. Viability is determined based on the absorbance difference between treated and untreated cells. Control refers to untreated cell receiving same volume of medium. The control did not demonstrate any inhibition. Percentage of inhibition increase with concentration of sample. The percentage of inhibition for concentrations 6.25, 12.5, 25, 50, 75 and 100 are 3.24, 9.07, 13.41, 34.80, 42.87 and 65.29 respectively (Table 2, Figure 4, Figure 5). 10 µl Ethanol is the vehicle control, which has 0.51% inhibition. IC50 value was calculated based on dose-response curves between the extract concentration and percentage of growth inhibition using the Graph Pad Prism 5 Software. IC50 value is equal to 79.44 µg/ml. [11]

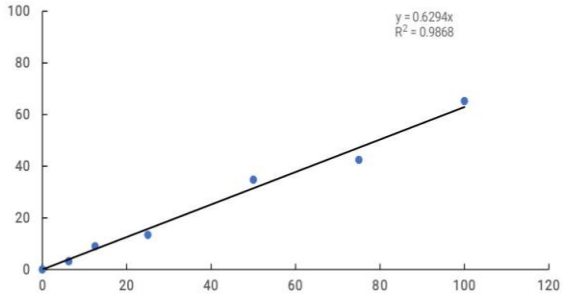

Figure 4 Graphical Representation of Concentration Against % Of Inhibition in MTT Assay

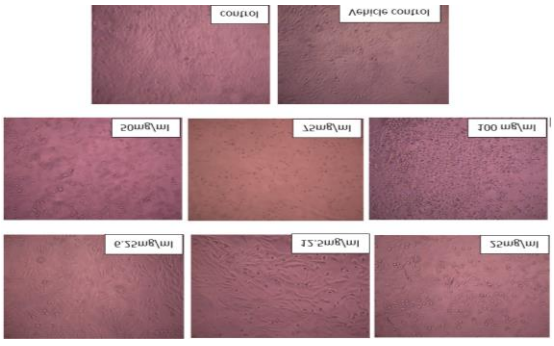

Figure 5 Mouse Breast Cancer Cell Line (C127I) Indicating Anticancer Property of Gardenia Resinifera Shoot Tip Exudate at Different Concentrations

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Table 2 The Percentage Inhibition of Proliferation Mouse Breast Cancer Cell Line (C127I) By Gardenia Resinifera Shoot Tip Exudate

		Absorbance							
	Con.(µg/ml)	I	II	III	IV	V	VI	Mean	%inhibition
Control	0	0.668	0.673	0.687	0.692	0.698	0.725	0.691	0
	6.25	0.64	0.646	0.679		0.686	0.692	0.669	3.24
	12.5	0.606	0.598	0.616	0.637	0.635	0.678	0.628	9.07
	25	0.586	0.615	0.576	0.606	0.61	0.597	0.598	13.41
	50	0.4	0.443	0.445	0.451	0.464	0.5	0.451	34.80
	75					0.394	0.401	0.398	42.47
	100	0.233	0.237	0.234	0.245	0.238	0.252	0.240	65.29
Vehicle control	(10µl Ethanol)	0.683	0.692					0.688	0.51

As per the US National Cancer Institute (NCI) plant screening programme, a crude plant extract is generally considered to have acceptable in-vitro cytotoxic activity if the IC50 value is less than 20µg/ml after incubation of 48- and 72-hour treatment of cancer cell line (Boik 2001). GC-MS analysis led to identification of five major components in shoot tip exudate. The detected components were GAMMA-MUUROLENE, Caryophyllene, ALPHA-CARYOPHYLLENE, NEOPHYTADIENE and Andrographolide. Components, retention time, percentage of area, height, percentage of height and their base m/z ratio are presented in Table 3 and Chromatogram1. Chromatogram is constructed with retention time in X axis and abundance of the signal in Y axis. There are several peaks labelled with their respective retention time. Each peaks represents the signal created when a compound elutes from the GC column into the detector. Greater peak size refers to the higher concentration of the component in the sample. Caryophyllene in GC-MS spectrum shows higher peak area and caryophyllene was already reported with its anti-inflammatory property isolated from Cinnamon (Tung et al. 2010). Some other reports using essential oils isolated from different Gardenia species also indicated the effectiveness of anti-inflammatory property of bioactive compounds in that essential oil (Zhang et al. 2022). Table 3 Shows Components of Gardenia Resinifera Shoot Tip Exudate Identified in GC-MS Analysis [13-15]

Table 3 Components of Gardenia Resinifera Shoot Tip Exudate Identified in GC-MS Analysis

Peak	R. Time	Area	Area%	Height	Height%	Name	Base m/z
1	16.000	705300	6.19	274970	7.14	GAMMA.-MUUROLENE	161.10
2	17.096	7150585	62.74	2602615	67.56	Caryophyllene	93.10
3	17.971	1380690	12.11	497479	12.91	ALPHA-CARYOPHYLLENE	93.10
4	26.553	277942	2.44	84760	2.20	NEOPHYTADIENE	68.10
5	48.558	1882039	16.51	392746	10.19	Andrographolide	109.10
		11396556	100.0	3852570	100.00		

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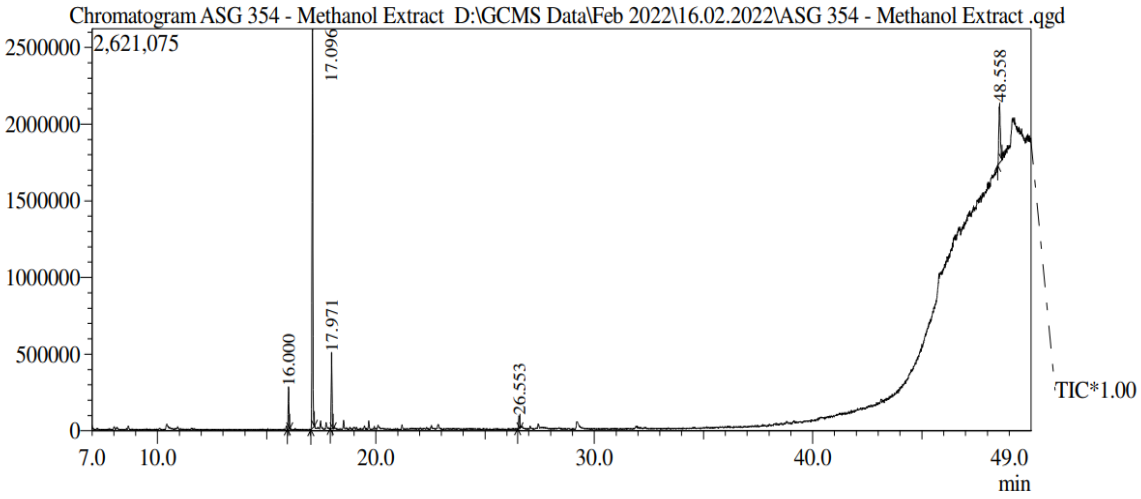

Figure 3 Chromatogram of Gardenia Resinifera Shoot

tip exudate indicating the presence of five different phytochemicals Caryophyllene has greater peak height of 67.56% and NEOPHYTADIENE has the least [17]. NEOPHYTADIENE has 2.20% peak height. Gamma- muurolene, Alpha- Caryophyllene and Andrographolide have peak height percentage of 7.14,12.91 and 10.19 respectively. Figure 3 shows Chromatogram of Gardenia Resinifera ShootConcentration of the components are as follows:

- Neophytadiene<Gamma
- muurolene<Andrographolide<Alpha
- Caryophyllene<Caryophyllene

Conclusion

The current study validates the anti-inflammatory and anticancer potential of Gardenia resinifera shoot tip exudate. The crude extract of shoot tip exudate was very effective in preventing the action of Cyclooxygenase and Lipoxygenase enzyme during progressive inflammation development. The study also disclosed the effectiveness of the shoot tip exudate in regulating the tumour cell development there by indicating its potential as an anticancer agent in cancer related cytotoxic drug development. GC-MS analysis explained the reason for this bioactive property of the exudate due to the presence of valuable bioactive components. Further separation and characterisation of these components will add valuable information for the development of highly efficient therapeutic drug in cancer research.

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