Dedicated to….

My mother

Late Buddhi Maya Rai

And

father

Late Shiba Kumar Rai

DECLARATION

I hereby declare that the thesis entitled "**Biological evaluation of indigenous medicinal plants from Darjeeling Himalayan region of West Bengal for natural compound(s) as inhibitor of cancer cell proliferation and studies on their molecular mechanism"** is a genuine research work carried out by me in the Department of Biotechnology, University of North Bengal, Darjeeling- 734013, West Bengal, India, under the supervision of **Prof. (Dr.) Shilpi Ghosh** and Co-supervision of **Dr. Anoop Kumar,** Department of Biotechnology, University of North Bengal, Darjeeling- 734013, West Bengal, India. I also affirm that the thesis is the original work and has not been submitted before in part or full for any degree, diploma or any other academic award to this or any other University or Institution.

Place: NBU

Date: 08/09/2023

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Prof. (Dr.) Shilpi Ghosh

CERTIFICATE

This is to certify that Mrs. Vijeta Rai has carried out her research work under our joint supervision with Dr. Anoop Kumar, Advanced Nanoscale Molecular Oncology Laboratory, Department of Biotechnology, NBU. Her thesis entitled**"Biological evaluation of indigenous medicinal plants from Darjeeling Himalayan region of WestBengal for natural compound(s) as inhibitor of cancer cell proliferation and studies on their molecular mechanism"** is based on her original work and is being submitted for the award of Ph.D. degree in Biotechnology in accordance with the rules and regulations of the University of North Bengal.No part of this thesis has formed the basis for the award of any degree or fellowship previously.

Date: 8/9/2023

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Dr. Anoop Kumar

CERTIFICATE

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Biological evaluation of indigenous medicinal plants from Darjeeling Himalayan region of West Bengal for natural compound(s) as inhibitor of cancer cell proliferation and studies on their molecular mechanism A Thesis submitted to the University of North Bengal For the Award of Doctor of Philosophy in Biotechnology By Vijeta Rai Under the supervision of Prof. (Dr.) Shilpi Ghosh Co – Supervision of Dr. Anoop Kumar DEPARTMENT OF BIOTECHNOLOGY UNIVERSITY OF NORTH BENGAL September, 2023

1 Chapter 1 General introduction and review

2 Chapter 1 General introduction 1.1. General introduction Natural products have played a significant role in drug development over the past century, with many drugs derived from natural sources. In fact, approximately 50% of the drugs approved by the U.S. Food and Drug Administration (FDA) between 1981 and 2010 were either natural products or derivatives of natural products (Majolo et al., 2019). Natural products are compounds that are produced by living organisms, including plants, animals, fungi, and microorganisms. These compounds have evolved over millions of years to perform specific functions in their native organisms, such as defense against predators or competition for resources (Zhang et al., 2005). The diversity of natural products is vast, and they offer a vast array of chemical structures and biological activities that can be harnessed for drug discovery. For example, alkaloids such as morphine and quinine are derived from plants and have been used for centuries to treat pain and malaria, respectively (Wase et al., 2008, Chopra et al., 2021). More recently, the anti-cancer drug paclitaxel was derived from the Pacific yew tree, and the anti-malarial drug artemisinin was isolated from the sweet wormwood plant. Natural products offer several advantages for drug development. They often have unique chemical structures and complex molecular frameworks that are difficult to synthesize, making them valuable starting points for drug discovery (Li et al., 2017; Grygorenko et al., 2020). They also have a proven track record of safety, as many of these compounds have been used in traditional medicine for centuries. Moreover, natural products offer a vast reservoir of novel chemical structures and biological activities that can be explored for drug discovery. With the increasing availability of genomic and metagenomic data, scientists are discovering new natural products from previously unexplored sources, such as plants, marine microbes and soil bacteria (Milshteyn et al., 2014). Ethnomedicine is the study of traditional knowledge and practices related to the use of plants and other natural resources for healing purposes. It involves the use of plants, animals, minerals, and other natural substances in the prevention and treatment of diseases. Ehnomedicinal plants play a crucial role in the search for new natural products with pharmacological properties (Sheng et al., 2001; Mustafa et al., 2017). Many traditional medicines are derived from plant-based sources, and a significant number of modern drugs have their origins in natural products. Ethnobotanical studies have identified numerous plants with medicinal properties, including anti-inflammatory, antimicrobial, anticancer, antidiabetic, and antihypertensive properties, among others. These plants have been used in traditional medicine systems for centuries and are still used today in many parts of the world. The use of ethnomedicinal plants is an important source of new natural products for

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PREFACE

The discovery of novel therapeutic agents from natural sources has always been an area of great interest in the field of medicinal research. Indigenous medicinal plants have been an integral part of traditional medicine systems, offering a vast repertoire of bioactive compounds with potential therapeutic properties. In recent years, there has been a growing interest in exploring the diverse flora of the Darjeeling Himalayan region of West Bengal, India, for the identification of natural compounds that could inhibit cancer cell proliferation.

Cancer remains a major global health concern, with a high mortality rate and limited treatment options for certain types of malignancies. Conventional cancer therapies often come with significant side effects and limited efficacy, prompting researchers to explore alternative approaches. Natural compounds derived from medicinal plants have shown promise as potential sources of anticancer agents due to their diverse chemical structures and biological activities.

The Darjeeling Himalayan region is characterized by its unique biodiversity, comprising of a wide array of plant species with medicinal properties. These plants have been traditionally used by local communities for various health-related purposes, including the treatment of cancer. However, the scientific validation of their efficacy and elucidation of their underlying molecular mechanism is essential for their integration into mainstream medicine. Therefore, research work presented herein aimed to evaluate the biological activity of indigenous medicinal plants from the Darjeeling Himalayan region, focusing on their potential as inhibitors of cancer cell proliferation. The study involved the collection and identification of plant samples, followed by their screening for phytochemicals, and antimicrobial and antioxidant activities, and cytotoxicity to cancer cells. A cytotoxic compound with steroidal skeleton was purified and characterized from *A. rivularis* rhizome and its mode of action on cancer cells was studied through in vitro inhibition of DNA synthesis enzyme, morphological analysis, and apoptotic gene expression analysis. The findings of this study have the potential to contribute to the development of novel therapeutic strategies for cancer treatment, utilizing the rich medicinal plant resources of the Darjeeling Himalayan region. The identification of bioactive compounds with potent anticancer properties and a better understanding of their molecular mechanisms will not only validate the traditional use of these plants but also offer new avenues for drug discovery and personalized medicine.

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