Declaration

I, Anindita Chakraborty hereby declare that the work embodied in my thesis entitled "Antileishmanial and Antifungal activities of Ethno-Medicinally important plant extracts used by Tribal populations of North Bengal" has been carried out by me under the supervision of Dr. Dipanwita Saha, Professor, Department of Biotechnology, University of North Bengal for the award of the degree of Doctor of Philosophy in Biotechnology. I also declare that, this thesis or any part thereof has not been submitted for any other degree/ diploma either to this or other University.

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TO WHOM IT MAY CONCERN

This is to certify that Ms. Anindita Chakraborty has worked under my supervision at the Department of Biotechnology, University of North Bengal. Her thesis entitled "Antileishmanial and Antifungal activities of Ethno-Medicinally important plant extracts used by Tribal populations of North Bengal" *is based on her original work and is being submitted for the award of Doctor of Philosophy (Science) degree in Biotechnology in accordance with the rules and regulation of the University of North Bengal. She has fulfilled all requirements according to the rules of the University of North Bengal regarding the works embodied in her thesis. This thesis or any part thereof has not been submitted for any other degree/Diploma either to this or other university.*

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Preface

India is a mega diversity country having numerous species of flora and fauna. Due to their proximity to rich natural resources, Indians have enriched their knowledge about the natural world, and this knowledge is also reflected in their daily life including treatment of illness. As such, we have a long heritage of using plants or plant products for medicinal and aromatic purposes. This study is a step to explore that age-old knowledge for the betterment of human civilization.

The essence of true India comes alive when we come to Dooars because of its rich biodiversity and vibrant rural life. The name Dooars is derived from 'doors' as the region is the gateway to the entire North-East India and Bhutan. The Dooars region which cover a vast area of 8800 sq. km. is famous for its rich wildlife and forests; the most notable of which are Gorumara National Park, Jaldapara Wild Life Sanctuary, and Buxa Tiger Reserve. The region is characterized by a sub-tropical and humid type of climate. The average maximum temperature is 37°C and the average minimum temperature is 6°C. The average annual rainfall of this area is 3300mm. The strong rain is of hydro-meteorological significance, causing deluges and flood in the area. The average relative humidity of this region is about 82%.

Almost all the forest villages and remote areas of this region are inhabited by various tribal populations. The local people use various plant species to cure common diseases, which became evident during a preliminary survey that involved direct interaction with tribal medicine men and healers practicing herbal medicines in their original primitive forms. Still, there are a large number of tribal areas having no healers or medicine men. Indigenous or traditional knowledge of human health and medicine can form a strong base of primary health care. There has been renewed interest

in plant medicine for the treatment of different diseases, as herbal drugs are generally devoid of toxic side-effects (Sharma et al., 2013). Studies show that different primary and secondary metabolites of systematic pathways of plants, mainly alkaloids, flavonoids, tannins, and phenolic compounds produce definite therapeutic actions on the human body. Knowledge of chemical constituents of phytoextracts is desirable for understanding their mode of action as therapeutic agents, and in the field of new drug discovery. At present time, phytochemical research based on ethnopharmacological information is the most effective and emerging approach to the world of medication.

Not only as curative agents, traditionally natural products, plants, vegetables, and fruits are also being used as tools for the prevention of diseases, infections and also for improvement of nutritional status. In today's world, natural antioxidants are a matter of great interest, and consumption of green tea, green vegetables, and organic foodstuffs have become a trend to overcome oxidative stress and delay aging. Research says there is an inverse relationship between intake of natural antioxidant-rich food and incidents of diseases in humans. Plants, having good antioxidant properties, are reported to have phenolic content of up to 30% of their dry weight. Many such antioxidant compounds have been isolated in recent studies (Lin et al., 1998; Rio et al., 2013).

Researchers of the modern age are concentrating their efforts to develop a new line of treatment and are trying to isolate novel molecules from natural resources to fight against the diseases which become a real threat to mankind. Chemical medicines and artificial drugs are proven to be less effective, with lots of side effects, which are used for diseases like cancer, microbial infection, systematic disorder, etc. Co-infection of two or more diseases, the evolution of new strain of microbes with different pathogenic characteristics, environmental changes and many other factors are

making the situation adverse day by day. Here lies the importance of cost-effective natural remedies with fewer or no side effects. Leishmaniasis is an example of a protozoan disease affecting millions of people in tropical countries with no proper line of treatment and effective vaccine. Moreover, in the last few decades, reports of co-infection of AIDS and Visceral leishmaniasis are making the situation worse. Visceral leishmaniasis (VL) accelerates the onset of AIDS in HIV positive people, and the chances of VL rise by 100-1000 times in immune-compromised patients (Fuzibet et al., 1988; Lindoso et al., 2009). This is a potentially fatal tropical disease, whose study is currently being neglected (de Paula et al., 2019), is considered by the World Health Organization (WHO) as the second most important protozoan disease in regard to public health (annual report 2009. Geneva, WHO).

Manifestation of leishmaniasis ranges from mild cutaneous lesions to fatal visceral form if left untreated. The first line of treatment, based on antimonial drugs, becomes useless when antimonial resistant *Leishmania* strains emerge and frequent relapses occur after treatment. Amphotericin β based second line of drugs also has severe limitations due to their toxic effects and high costs. Despite continuous efforts by scientists to develop a vaccine, no one has yet been protected against *L. donovani*. Visceral leishmaniasis attacks the immune system of the host, characterized by defective cell-mediated immunity, increase in cell membrane fluidity, hampering of antigen-presenting ability and also impairment of specific T-cell response (Chakraborty et al., 2005). In such immuno-compromised condition, other opportunistic microbes like fungi (*Candida sp., Cryptococcus sp., Aspergillus sp.* etc.) can easily attack humans, making the situation complicated. Immuno-modulator drugs can improve the situation by activating the microbicidal mechanism of macrophages and by stimulating the host's overall immune system. Studies say many plant extracts or natural products have immuno-

modulatory activities. In this scenario, it is necessary to develop a novel line of treatment having direct and selective microbicidal effects – which can eradicate age-old diseases like leishmaniasis and related complications. In the humid and wet weather of sub-Himalayan West Bengal, fungal infections are most common cause of skin diseases. Hepatomegaly and splenomegaly are very common among these people. During the preliminary survey through local people, it was found that they use particular herbs to cure skin diseases, liver disorders, and diseases like leishmaniasis (Mitra and Mukherjee, 2009; Mitra and Mukherjee, 2010). This potential of the herbs can be explored to discover bioactive molecules relating to new drug development against leishmaniasis and fungal infections.

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ABBREVIATION

ABCD	Amphotericin beta lipid complex
APC	Antigen Presenting cell
BSA	Bovine serum albumin
BSI	Blood stream infection
CL	Cutaneous leishmaniasis
Cm	Centimetre
CPG	Cytosine-phosphate guanosine
DCL	Diffuse cutaneous leishmaniasis
Dia	Diameter
DMSO	Dimethyl sulfoside
DPPH	2, 2'-diphenyl-1-picryl Hydrazyl Radical
EDTA	Ethylenediaminetetraacetic acid
ELISA	Enzyme-linked immune sorbent assay
ER	Endoplasmic reticulum
EtoAc	Ethyl acetate
FBS	Fetal bovine serum
FCS	Fetal calf serum
$FeCl_3$	Ferric chloride
FML	Fructose mannose ligand
gp63	Glycoprotein 63 kd
H_2O_2	Hydrogen peroxide
HASPBI	Hydrophilic acylated surface protein beta1
HCl	Hydrochloric acid
HIV	Human Immuno-deficiency Virus
HOCL	Hypochlorous acid
Hr	Hour
IFAT	Indirect fluorescent antibody test
IFNγ	Interferon gamma
Ig	Immunoglobulin
IgG	Immunoglobulin G
IL	Interlukein
IVDU	Intervenous drug user
kDa	Kilo Dalton
kDNA	Kinetoplastid DNA
Kg	Kilogram
Km	Kilometre
LAMB	Liposomal amphotericin beta

LD	*Leishmania donovani*
LPCB	Lactophenol cotton blue
LPG	Lipophosphoglycan
M	Molar
MCL	Mucocutaneous leishmaniasis
MeOH	Methanol
Mg	Milligram
MHC	Major Histo-compatibility Complex
Min	Minute
mL/ ml	Milliliter
mm	Millimetre
µg	Microgram
µl	Microlitre
µm	Micrometre
mM	Millimolar
Mmol	Millimole
MTCC	Microbial type culture collection
MTT	3(4,5dimethylthiazole-2-yl)-2,5 diphenyl
N	Normal
NAC	Non albican species
13C NMR	Carbon nuclear magnetic resonance
1H NMR	Hydrogen nuclear magnetic resonance
OD	Optical density
PBMC	Peripheral blood mononuclear cells
PBS	Phosphate buffer saline
PDA	Potato dextrose agar
PDB	Potato dextrose broth
PEC	Peritoneal exudates cells
Pet ether	Petroleum ether
PKDL	Post Kala-azar Dermal Leishmaniasis
ROS	Reactive oxygen species
Rpm	Rotation per minute
S	Seconds
SAG	Sodium antimony gluconate
SD	Standard deviation
SE	Standard error
SGOT	Serum glutamate oxaloacetate transaminase
SGPT	Serum glutamate pyruvate transaminase
SOD	Superoxide dismutase

sp.	Species
SSG	Sodium stibo gluconate
TC	Total count
TGFβ	Transforming growth factor beta
Th	T helper
Th-1	T helper-1 cells
Th-2	T helper-2 cells
TLC	Thin layer chromatography
TNFα	Tumor necrosis factor alpha
UV	Ultraviolet
v/v	Volume by volume
VL	Visceral leishmaniasis
Vol.	Volume
VV	Vaccinia virus
WBC	White blood cell
w/v	Weight by volume
WHO	World health organization
ZOI	Zone of Inhibition