Evaluation of Toxicity, Antioxidant, Antidiarrheal activity and Phytochemical Screening of methanolic extract of leaf and stem of *Actephila excelsa*

A project submitted

by

Tahsina Ani

ID: 14146033

Session: Spring 2014

to

The Department of Pharmacy

In partial fulfillment of the requirements for the degree of

Bachelor of Pharmacy (Hons.)

Dhaka, Bangladesh

September 2018

This humble effort is dedicated to my family for their affection and encouragement along with my honorable supervisor Md. Samiul Alam Rajib for his continuous support

Certification Statement:

This is to certify that this project titled “Evaluation of Toxicity, Antioxidant, Antidiarrheal activity and Phytochemical screening of methanolic extract of leaf and stem of *Actephila excelsa*” submitted for the partial fulfillment of the requirements for the degree of Bachelor of Pharmacy (Hons.) from the Department of Pharmacy, BRAC University constitutes my own work under the supervision of Md. Samiul Alam Rajib, Senior Lecturer, Department of Pharmacy, BRAC University and that appropriate credit is given where I have used the language, ideas or writings of another.

Signed,

_____

Countersigned by the supervisor,

_____

Acknowledgement

First and Foremost, I am very thankful to Almighty Allah for granting me the noble opportunity to accomplish my project and endowing me with lots of patience and strength throughout the period.

Secondly, I owe my immense gratitude and honor to my respected supervisor Md. Samiul Alam Rajib, senior lecturer, Department of Pharmacy, BRAC University, who continually and convincingly bestowed the courage and confidence upon me for the completion of the project properly. Without his dedicated involvement, persistent support, suggestions and kind encouragement the project would not have been accomplished successfully.

I cannot find words to express my heartiest gratitude to Professor, Dr. Eva Rahman Kabir, chairperson, Department of Pharmacy, BRAC University for her sincere guidance and continuous superintendence during the period.

I further like to thank all the faculty members of Department of Pharmacy, BRAC University for their support and motivation throughout the time as well as the lab officers for their assistance and help.

Most importantly, none of these could have happened without my beloved family. Their unwavering inspiration and support for this long period help to complete this project smoothly.

Abstract:

For a long time, human is seeking thoroughly for natural medicines to treat different types of diseases. Disregarding the expansion of synthetic drugs to treat incessant disease, the exploitation of natural medicines keeps on thriving. The plant *Actephilaexcelsa*belongs to the family *Phyllanthaceae*. For this research, stem and leaf of *Actephila excelsa* were preferred to evaluate its antioxidant, antidiarrheal properties as well as the toxicological activities and phytochemical screening. The primary reason behind choosing this plant is that the dried out leaves of the plant are commonly used to make tasty tea. So, it could be rich with phytochemicals and antioxidants. The secondary reason is that no study still has been carried out to identify its biological properties. In this study, to establish the antioxidant properties in-vitro DPPH free radical scavenging, total phenolic content and total flavonoid content determination were carried out. Antidiarrheal (castor oil and $MgSO_{4)}$ and toxicological activities were performed *in vivo* using Swiss albino rats. The study defined strong DPPH scavenging activity and total phenolic content but mild flavonoid content. The phytochemical constituents (Carbohydrates, alkaloids, flavonoids, tannins, saponins) were present. The leaf extract showed high antidiarrheal potentials. It showed significant difference in various organ parts for both leaf and stem extracts. In contrast, no significant difference ($p < 0.05$) was noticed in body weights. Significant difference was also observed in WBC, RBC, platelets, neutrophil, lymphocytes, monocytes, PDW, SGPT, SGOT, cholesterol level while performing the histopathological and biochemical examinations. Therefore, after performing the toxicological test it can be decided that the plant is toxic to be used for long period.

Table of Contents **Page no**

List of tables:

List of Figures:

List of abbreviations:

SOD = Superoxide dismustase

CAT = Catalase

GPX = Glutathione peroxidase

ESR = Erythrocyte sedimentation rate

PCT = Procalcitonin

ALT = Alanine aminotransferase

AST = Aspertate transaminase

IC_{50} = Median Inhibitory Concentration

LC_{50} = Median Lethal Concentration

ASA = Ascorbic acid

WHO = World Health Organization

MCHC = Mean corpuscular hemoglobin concentration

DPPH = 2, 2-Diphenyl-1-Picrylhydrazyl

Hct = Hematocrit

MCV = Mean corpuscular volume

MCH = Mean corpuscular hemoglobin

PDW = Platelet distribution width

RDW = Red cell distribution width

MPV = Mean platelet volume

Chapter 1

Introduction

1.1 Introduction:

Humans, for their basic needs are dependent on plants such as for the production of foods, shelter, transportation, furniture, clothing, fragrance and most importantly for medicines. Furthermore, they play considerably vital role in regulating the hydrological cycle and amount of oxygen and carbon di oxide in the atmosphere. The term “Medicinal plants” are considered to the herbs which are generally utilized for the management of diverse diseases and considered to play significant role in healthcare(Sadi, 2012). Herb is defined as any fraction of the plant like seed, flower, bark, tree, leaf, root, stem, stigma and also non-woody plants. The medicinal plants or herbs are broadly utilized in the developing country, where in numerous spots they offer a more generally accessible and more moderate modification to pharmaceutical medications. In Africa, for instance, up to 80% of the population relies upon them, as indicated by WHO(Greenwood & Wise, 2001). Besides, the pharmacological, therapeutical significance, these have economic significance as well. Medicinal plants are cost effective and more easily accessible for those people who have good ideas about it. The bioactive compounds derived from medicinal plants can are a source of great importance in pharmaceutical company for raw materials. So these are considered as asset to a country’s healthcare system. More than 500 medicinal plants are present in our country that have well-known scientific claim (Ghani, 2003). In ancient times, there was lack of information concerning the causes of illness and which particular plant could be utilized in which way for a particular disease. Gradually, the explanations of the utilization of definite restorative plants to treat specific illnesses were being established; thus, the beneficial uses of plants are progressively being popular and stimulated on the way to becoming conventional on instructive certainties (Petrovska, 2012).Ever since times millions of plants are acclimated to treat illness including epidemics and assure improved physical condition in humans. The information of these properties of medicinal plants spread among human groups throughout hundreds of years. Medicinal plants are known as the “spine” of traditional medicine because over 3.3 billion people use traditional medicines to cure disease on a regular basis in the less developed countries(Davidson-hunt, 2000).The use of therapeutic plants in treatment is seen as extraordinarily ensured as these hold no or unimportant adverse effects. The utilization of medicinal plants for drug development in developing nations, for

supporting the welfare of people, has been generally seen by UNESCO. The modern scientific medicines are treading on with the advancement of civilization. Despite of this, traditional medicine is the primary concern of majority of people to cure illness in industrialized and developing countries. The proportion is drastically increasing because of its safety issues, quality and efficacy. Also, the general people choose traditional medicine over modern scientific medicine for historical and cultural reasons(Ajlan, 2016). Different parts of medicinal plants are continuously used forthe extraction of active ingredients followed by synthesis of different important drugs like antibiotics, anticancer, blood thinners, laxatives, antioxidants, and anti-malarial medications. Many plants include a number of secondary metabolites like alkaloids, saponins, phenols, essential oils, flavonids, tannins, quinines, steroid and the active ingredients of Taxol, vincristine, and morphine. Previous works clearly demonstrated that the phenolic compounds have potentially important effect on biological actions such as antioxidant, ant-inflammatory and antifungal actions(Brewer, 2011). Medicine, in a number of developing nations, using confined traditions and viewpoint, is the foundation of health care still now. As defined by WHO, health is a status of entire physical, social, and mental well-being and not just the nonexistence of ailment or disability. So, medicinal plants can construct an imperative role to the goal of WHO to make sure that people worldwide, will be capable of leading a sustainable socio-economic dynamic life by the year 2000 (Singh, 2015).

1.2 History of medicinal plant:

There is no composed record of the ancient people, their ailments, or their medicinal medications; notwithstanding, information that was compiled together from this time by researchers and historians give an essential understanding of ancient individuals' health and lifestyles (Stojanoski, 1999). Numerous of the mending techniques that were produced by individuals in all parts of the world were more comparative than might be expected, considering the wide and assorted geographic zone that was possessed by ancient individuals. The most established composed proof of restorative plants' use for planning of medications has been originated from a Sumerian earth piece from Nagpur, around 5000 years of age. That involved 12 formulas for production of drug alluding to more than 255 different plants; few of those are alkaloids, for example, poppy, mandrake and henbane

(Kelly, 2009) .The Chinese book composed by Emperor Shen Nung entitled "Pen Ts'ao" depicted 365 medications, a considerable lot of which including yellow gentian, ginseng, cinnamon bark, Theae folium, camphor, podophyllum, jimson weed, furthermore, ephedra are utilized even today(Wiart, 2006).

There are a few other age-old books starting from different nations. However, the most noticeable one is from the "father of pharmacognosy," Dioscorides which offers considerable information on restorative plants constituting the fundamental materia medica until the resurgence (Thorwald, 1999). It depicts in excess of 1000 medications, 66% of them being of natural source and it clarifies their method of gathering, and restorative impacts. In this book, other than plant depictions, the plant names in different dialects and the territories where they happen are given. The fifteenth to the seventeenth century was a thriving period for herbal medicines and their portrayals started to show up in a few dialects. In the eighteenth century, Linnaeus concocted a scheme for grouping plant species (Dwivedi, 2008) With a specific end goal to name plants, a polynomial framework was utilized and there the principal word indicated the genus whereas staying polynomial words clarified different highlights of the plant The early nineteenth century was a defining moment in the learning base, and also the utilization of medicinal plants(Gruenwald, Brendler, & Jaenicke, 2000). In the late nineteenth and mid twentieth hundreds of years, there was an enduring decrease in the restorative utilization of natural pharmaceuticals. Numerous authors asserted that medications got from medicinal plants hold inadequacies principally due to the negative activity of catalysts. In addition, in the nineteenth century, glycosides and alkaloids, separated in unadulterated frame and utilized in therapeutics were progressively supplanting the natural medications from which they had been inferred (Swerdlow, Joel, 2000). In spite of the fact that the activity of unadulterated alkaloids was much faster, the impacts of plant-inferred alkaloidswere broad as well as durable. Right on time in the 20thcentury, stabilization processes for the arrangement and utilization of new medicinalplants were proposed. Besides, significant endeavors were put resources into the investigation of ideal conditions for developing and assembling restorative plants(Sewell & Rafieian-kopaei, 2014).As of now, because of the strong side effects of synthetic drug and expanding contraindications to their utilization, a famous resurgence has appeared for the

utilization of therapeutic plants.Because of different clinical, chemical and physiological investigations, various overlooked plants or medications got thereof were reestablished in drug store: *Hyosciamus, Opium, Stramonium, Secale cornutum, Styrax, Punica granatum, Colchicum, Ricinus*, and so on. The human life form acknowledges the medication got from them best ashuman is an indispensable piece of nature(Nelson & Cox, 2005).All through the medieval times, European doctors counseled Persian and Arab works, for example, "De Re Medica" by John Mesue, "Standard Medicinae" by Avicenna, and "Liber Magnae Collectionis Simplicum Alimentorum et Medicamentorum" by Ibn al-Baitar and there over 1000 plants of medical importance were recognized. Marco Polo's ventures (1254-1324) in tropical Asia, Persia and China, the revelation of America (1492), and Vasco De Gama's trips to India (1498), brought about the exchange of numerous restorative plants to Europe. Amid this period, Paracelsus (1493-1541) was a main defender of synthetically arranged medications from crude plants and mineral substances (Nelson & Cox, 2005).These days, herbal medicines get noticeable values in all the pharmacopeias of the world. Truth be told, nations, for example, Germany, the United Kingdom and Russia really have isolated herbal pharmacopeias. In any case, as a general rule, there is a higher occurrence and more across the board utilization of informal herbal drugs. Their application is grounded either in traditional medicines or on current scientific researches(Jacquart, 2008). Numerous medicinal plants are utilized as self-solutions or then again are utilized upon the suggestion of a doctor or pharmacist. They are utilized both autonomously and potentially in mix as correlative medications to manufactured medications. It is basic for a satisfactory furthermore, effectively connected treatment that an exact early determination is made of the ailment alongside determination of the proper pharmacological impacts related with the particular home grown segments(Nelson & Cox, 2005).

1.3 Importance of medicinal plants:

- Many plants attribute in blood purification to alter a long-standing state by removing the metabolic poisons. Some specific plants improve the immunity of the human being, and thus improve the conditions. For example, fever(Kritikar & Basu, 1918).

- Because of the decreasing of heat and the decrease in temperature due to fever, Ayurvedic practitioners recommend specific plants such as pepper, sandalwood etc.(Kritikar & Basu, 1918).
- An important supplement to diet is to act as an antiseptic.Various medicines can be directly or indirectly produced by the plants having medicinal value. For example, aspirin(Singh, 2015).
- To neutralize acid produced by the stomach, herbs such as root and marshmallow leaf are used. They are really good source of antacids. The stomach acid necessary for good digestion is maintained by such herbs (Srivastava, 2018)**.**
- Turmeric plays important role in inhibiting the growth of germs and bacteria, harmful microbes. Turmeric remedy is widely used to treat injuries at home. Ginger, Cardamom, cloves are commonly used as they possess expectorant property, which liquefy the mucus of bronchi and promotes the ejection of it.
- To protect the biological diversity, it is highly recommended to cultivate and preserve medicinal plants. For example, themetabolic engineering of plants (Singh, 2015).
- Arka, katuka these herbs contain disinfectant property, which destroys germs that lead to various disease. We know that communicable diseases are caused by some pathogenic microbes. These herbs also kill those microbes by inhibiting their growth (Srivastava, 2018)**.**

1.4 Some medicinal plants in Bangladesh:

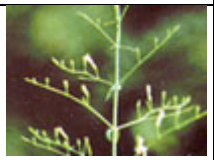
There are around 2000 plants were enrolled as therapeutic plant for including medicinal properties inside the Asian subcontinent and of them more than 722 plants develop in Bangladesh. As indicated by (Ghani, 2003), around 500 medicinal plants name was enrolled as their availability in Bangladesh.

Table: 1.1 Important medicinal plants in Bangladesh (Bardhan, Ashrafi, & Saha, 2018)

Medicinal Uses	Scientific names of the plants
Asthma	*Allium sativum, Ocimum sanctum, Piper nigrum, Abelmoschus moschatus*
Cough and dry cough	*Sesbania grandiflora, Vitex negundo, Zingiber officinale,Pistia stratiotes,Punica granatum*
Diabetes	*Trigonella foenum, Syzygium cumini,Tinospora cordifolia,Magnifera inica,Cajanus cajan,Abelmoschus esculentus,Catharanthus roseus*
Diarrhea	*Mimosa pudica, Nymphaea nounchali, Ocimum basilicum, Alium sativum, Phyllanthus emblica*
Headache	*Nigella sativa, Ocimum gratissimum, Ocimum sanctum,* Terminalia chebula
Hypertension	*Phylanthus emblica, Polyalthia longifolia,Rauvolfia serpentine,Withania somonifera*
Indigestion	*Mentha spicata, Nigella sativa, Strychnos nux-vomica, Syzygium cumini,Tamarindus indica*
Jaundice	*Phylanthus emblica, Phylanthus niruri, Ricinus communis,Spinacea oleracea;Tinospara cordifolia, Zingiber officinale*
Migraine	*Mimusops elaugi, Ocimum gratissimum, Piper longum, Sphaeranthus indicus ,Withania somnifera, Woodfordia fruticosa*

1.5 List of commonly used medicinal plants and their uses: (OFDC)

Table: 1.2 Commonly used medicinal plants, used parts and their uses:

Plant	Common name, Maturity period	Botanical name and family	Parts used	Medicinal uses
	Amla (T) After 4th year	Emblica officinalis Fam:euphorbiaceac	Fruit	Vitamin - C, Laxative Cough, hyper acidity Diabetes, cold.
	Ashok (T) 10 years onward	Saraca Asoca Fam:Caesalpinanceae	Bark Flower	Deiabetes, Menstrual Pain, uterinedisorder
	Aswagandha (H), One year	Withania Somnifera Fam: Solanaccac	Root, Leafs	Restorative Tonic, stress, nerves disorder, aphrodiasiac.
	Bael / Bilva (T)After 4-5 year	Aegle marmelous Fam: Rutaccac	Fruit, Bark	Diarrrhoea, Dysentry, Constipation.
	Chiraita (high altituted) within one year (H)	Swertia Chiraita Fam : Gentianaccac	Whole Plant	Skin Desease, Burning, censation, fever.
	Kalmegh/ Bhui neem (H) within 1 year	Andrographis PaniculataFam : scanthaccac	Whole Plant	Fever, weekness, release of gas.

	Long peeper / Pippali (C) after (2-3) years	Peeper longum Fam : Piperaccac	Fruit, Root	Appetizer, enlarged spleen, Bronchities, Cold, antidote.
	Sarpa Gandha (H)After 2 year	Ranwolfia Serpentina Fam: apocynaccac	Root	Hyper tension, insomnia.
	Tulsi (perennial) Each 3 months	Ocimum sanclum Fam: Lamiaccac	Leaves /Seed	Cough, Cold, bronchitis,expectorand.
	Pippermint (h) Perennial	Mentha pipertia Fam:Lamiaccac	Leaves, Flower, Oil	Digestive, Pain killer.
	Henna/Mehdi (S) 1/25 years	Lawsennia iermis Fam: lytharaceae	Leaf, Flower, Seed	Burning, Steam, Anti Imflamatary.
	Gritkumari (H) 2nd-5th yr	Aloe Verra Fam: Liliaceae	Leaves	Laxative, Wound healing, Skin burns & care,Ulcer.
	Neem (T)	Azardirchata - indica Fam : Mahaceae	Rhizome	Sdedative, analgesic, epilepsy, hypertensive.

1.6 Introduction to the selected plant: (*Actephilaexcelsa*)

1.6.1 Family of the selected plant: Phyllanthaceae

Phyllanthaceae is composed of 59 genera and in excess of 1,700 species of medicinal plants that develop in tropical areas; the family is particularly different in Malaysia. The species ofPhyllanthaceae family has regularly finely splitting bark and two-positioned leaves that

need organs. There are two seeds in each chamber in organic products. The pantropical Phyllanthus (about 1,300 species which also includes the previous genera Glochidion, Breynia, and Sauropus) has basic leaves that regularly appear as though they are pamphlets of a compound leaf. Glochidion (around 300 species) is tropical, barring terrain Africa, and species are especially basic in Indo-Malaysia. Sauropus (70 species) develops from Indo-Malaysia to Australia. Antidesma (100 species) is local to the Paleotropics and warm mild areas. Cleistanthus (140 species) is local to the Paleotropics. Baccaurea (50 species) develops from Indo-Malaysia toward the western Pacific. Aporosa (90 species) is local to Indo-Malaysia. Bridelia (50 species) develops all through the Paleotropics. Uapaca (60 species) is found in Africa and Madagascar (WHO monographs, 1999).

1.6.2 Taxonomy of Phyllanthaceae:

(Hoffmann, Kathriarachchi, & Wurdack, 2006)

Sub family	Tribe	Sub tribe
SubfamilyI. Phyllanthoidea	**Tribe 1.**Poranthereae	-------
	Tribe 2. Bridelieae	**Subtribe 2a**.Amanoinae **Subtribe 2b**.Saviinae **Subtribe 2c**. Keayodendrinae **Subtribe 2d**. Pseudolachnostylidinae
	Tribe 3.Wielandiea	**Subtribe 3a**.Astrocasiinae **Subtribe 3b**.Wielandiinae
	Tribe 4.Phyllantheae	----------

Subfamily II: Antidesmatoideae	**Tribe 5.**Antidesmateae	**Subtribe 5a**.Antidesmatinae **Subtribe 5b**.Hymenocardiinae **Subtribe 5c**.Martretiinae **Subtribe 5d**.Hieronyminae
	Tribe 6.Scepeae	----------
	Tribe 7.Jablonskieae	
	Tribe 8.Spondiantheae	
	Tribe 9.Uapaceae	
	Tribe 10: Bischofieae	

1.6.3 Major phytochemicals isolated from phyllanthaceae and their pharmacologic effect:

The real phytochemicals which have/may have a part in rendering the herbs with restorative properties are recorded in Table: 1.3 (Sarin, Verma, Martín, & Mohanty, 2014)

Table: 1.3 Major phytochemicals isolated from phyllanthaceae and their pharmacologic effect:

Species	Class	Phytochemicals	Pharmacologic effect	References
P. amarus			Anticancer antitumor Antileukemia	(Harikumar, Kuttan, Kuttan, 2009) (Mazumder, Mahato, & Mazumder, 2006) (LONDHE,

	Lignans	Phyllanthin	Antibacterial	DEVASAGAYAM, FOO, & GHASKADBI, 2009) (Recio, Giner, Máñez, & Rios, 1995)
			Antiamnestic Antiaging, Antioxidant Anti-inflammatory	(Krithika et al., 2009)
	Flavonoids	Rutin	Radioprotective Antioxidant	(Mao et al., 2016) (Gutiérrez M, Carmona U, Vallejos G, 2012)
	Tannins	Amariin	Antioxidant Radio protective Hepatoprotective	(Gutiérrez M, Carmona U, Vallejos G, 2012)(Mao et al., 2016) (Recio et al., 1995)
	Alkaloids	Norsecurinine	Antifungal	(Ali, Houghton, & Soumyanath, 2006)
	Volatile oil	Linalool, phytol	Antimicrobial Anticancer	(Gutiérrez M, Carmona U, Vallejos G, 2012)(Joshi, 2006)
P.fraternus	Alkamide	E,E-2,4-Octadienamide	Antiplasmodial	(Fang, Rao, & Tzeng, 2008)
P. debilis	Oxirano-furanocoumarin	Debelalactone	Antihepatotoxic	(Ali et al., 2006)

P. tenellus	Lignan	Niranthin Nirtetralin	Antiviral	(LONDHE et al., 2009)
P. urinaria	Lignan	Phyllanthin	Anti-inflammatory	(Shin, Kang, & Lee, 2005)
	Flavonoid	Quercetin	Anti-inflammatory	(Shin et al., 2005)
	Coumarin	Methylbrevifolin carboxylate	Antioxidant	(Shin, Kang, & Lee, 2005)
	Ellagitannin	Ellagic acid	Antiviral	(Islam, Selvan, Mazumder, & Gupta, 2008)
P. virgatus	Lignan	Niranthin	Antiviral	(LONDHE et al., 2009)
		Nirtetralin	Antiviral	(LONDHE et al., 2009)
	Ellagitannin	Geraniin	Antiviral	(LONDHE et al., 2009)

1.6.4 Botanical descriptions of *Actephila Excelsa*:(Sasidharan, 2004)

Habit : Shrub or little tree, height can be 8m

Bark : Brownish bark and the blaze are pink.

BranchesandBranchlets : The branchlets are glabrous

Leaves : The leaves are spiral, simple and alternate; a small leafy outgrowth at the base of a leaf or its stalk usually occurring in pairs.Petiole is about 2 cm, which is enlarged at both ends. Thelamina is 15 × 4 cm which is variable. The shape can be

either elliptic-oblanceolate or lanceolate.It contains attenuated base, 6-12 nerves (secondary) and some reticulated tertiary nerves.

Flower : The flowers are unisexual having male and female reproductive organs in separate plants. Male flowers make clusters in axillary axis; Pedicellates are short. These have white petals and yellow disk. Female flowers do not make any cluster having long pedicellates. The petals are small, yellowish and the disk is yellow.

Fruit & Seed: Generally, these are capsule, globose and compressed. Each cell contains 2 seed (2 ×3.2cm).

Distribution: Peninsular India, Sri Lanka, Indonesia, Malaysia, Myanmar, Philippines, Thailand, Vietnam.

Uses: The dried out leaves are used for making an amusing tasting tea.

It is also used in wound healing which is reported by the general people of Sylhet.

1.6.5 Taxonomical classification of *Actephila excelsa*

Kingdom: Plantae
Phylum: Tracheophyta
Class: Magnoliopsida
Order: Malpighiales
Family: Euphorbiacea or Phyllanthaceae
Genus: *Aectphila*
Species: *Actephila excelsa*

1.7 Related publications on *Actephila excelsa*:

There are no previous reports of publication on *Actephila excelsa* except the publication on chemical constituents of *actephila excelsa*. No experiments have yet been carried out on its antioxidant property, antidiarreal property, and toxicological activity. As a result, different important properties and medicinal values of the plant still essential to be recognized.

1.8 Purpose of the project:

The purpose of the project is to identify the important medicinal values of the selected plant for the greater welfare of the human society and establish different unknown biological properties.

1.9 Objective of the project:

The objectives of the project are as follows:

- Evaluation of the antioxidant properties of the methanol extract of the leaf and stem of plant*A. excelsa* by determining DPPH free radical scavenging activity, total phenolic content and total flavonoid content.
- To perform phytochemical screening test of the methanol extract of the leaf and stem of plant *A. exccelsa.*
- To find out the antidiarreal activity of the methanol extract of the leaf and stem of plant *A. excelsa.*
- Determination of the toxicological activity of the methanol extract of the leaf and stem of the selected plant.

1.10 Importance of antioxidant potential of medicinal Plants:

Antioxidant mainly inhibits the oxidation of oxidizable substrates(Sies, 1986). Antioxidants can both be taken as dietary supplement and also can be synthesized in vivo; for example:superoxide dismutase (SOD), lessened glutathione (GSH) etc (Sies, 1997). Studies have revealed that around 66% of the total plants of the world possess medicinal significance and also splendid cancer inhibitory agents. The eagerness for the plant

antioxidant wasaroused at first by revelation and ensuing detachment of ascorbic acid from different plants(Krishnaiah, Sarbatly, & Nithyanandam, 2011).From that point forward, the cancer prevention agent capability of plants has gotten a lot of consideration in light of the fact that expanded oxidative pressure has been renowned as a noteworthy contributory factor in the improvement and movement of a few hazardous ailments, including neurodegenerative and cardiovascular malady. Furthermore, exogenous antioxidantssupplement or augmented endogenous antioxidant resistances of the body are observed to perform as a favorable technique for refuting the disastrousinfluences of oxidative pressure(Kasote, Hegde, & Katyare, 2013).

There are as of now roughly ten in vivo and nineteen in vitro strategies for surveying this property which are usually connected for the assessment of the cancer inhibitoryagent's action of plants. In a large portion of these in vitro measures plant tests demonstrated strong antioxidant agent action. This is likely because of their inborn capacity to integrate non-enzymatic antioxidants, for example, glutathione, phenolic compounds, ascorbic acid and secondary metabolites(Chanda & Dave, 2009).

1.10.1 The reasons behind almost entire plants having antioxidant potentials:

Chloroplasts and the power houses are considered to be important sites to generate reactive oxygen substances in plant cells. Moreover, peroxisomes are considered to be the 3rdimportant sites for producing reactive oxygen substances (superoxide, peroxide, nitric oxide) within plant cells. The generation of reactive oxidative stress occurs at the site of photosystem I and II of the chloroplasts, peroxisome's matrix and membranes, complex I, complex III and ubiquinone of the electron transport chain of mitochondria (Gill & Tuteja, 2010). These are frequently created in the sub cellular organelles of cells. Though this production is planned genetically as they act as signaling molecules, overproduction may sometimes harm DNA, proteins and lipids(Zhao, Davis, & Verpoorte, 2005).The whole process of generating the free radicals is shown in the figure:

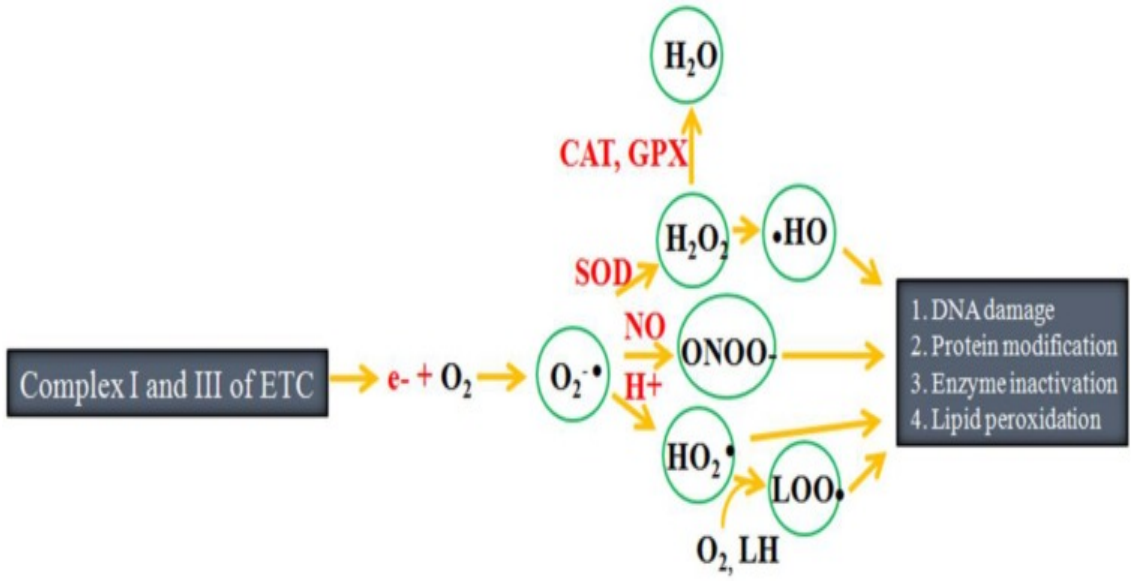


Figure: 1.1 Production of free radical (Carocho & Ferreira, 2013)

During transfer period of electrons, around one to two percentage of total electrons slip from the mentioned complex(I,III) of the electron transfer chain (ETC), later there is a reaction between molecular oxygen and electrons for the formation of different free radicals for example,

Lipid peroxyl radical ($LOO^{\bullet}$), hydroxyl radical, hydroperoxyl radical ($HO_2^{\bullet}$), peroxynitrite ($ONOO^-$) superoxide anion ($O_2^{-\bullet}$) and hydrogen peroxide (H_2O_2). These radicals then aim at DNA, lipids and protein and eventually destruct them.

Here, SOD stands for superoxide dismutase

CAT stands for catalase and

GPX stands for glutathione peroxidase

(Carocho & Ferreira, 2013)

Plants contain proficient complex system of both non-enzymatic and enzymatic antioxidant resistance mechanisms to evade the poisonous properties of free radicals. For the production and gathering of these non-enzymatic antioxidants there are two reasons. First, the genetic character of plant is organized in such a way that makes the plants capable of having a natural capacity to produce an extensive range of phytochemicals for carrying out their ordinary physiological roles and save them from different microbial substances. The second

reason could be the innate preference of the plants to react to environmental stress conditions (Abd El-Baky, El Baz, & El-Baroty, 2009). The figure illustrates the overall mechanisms:

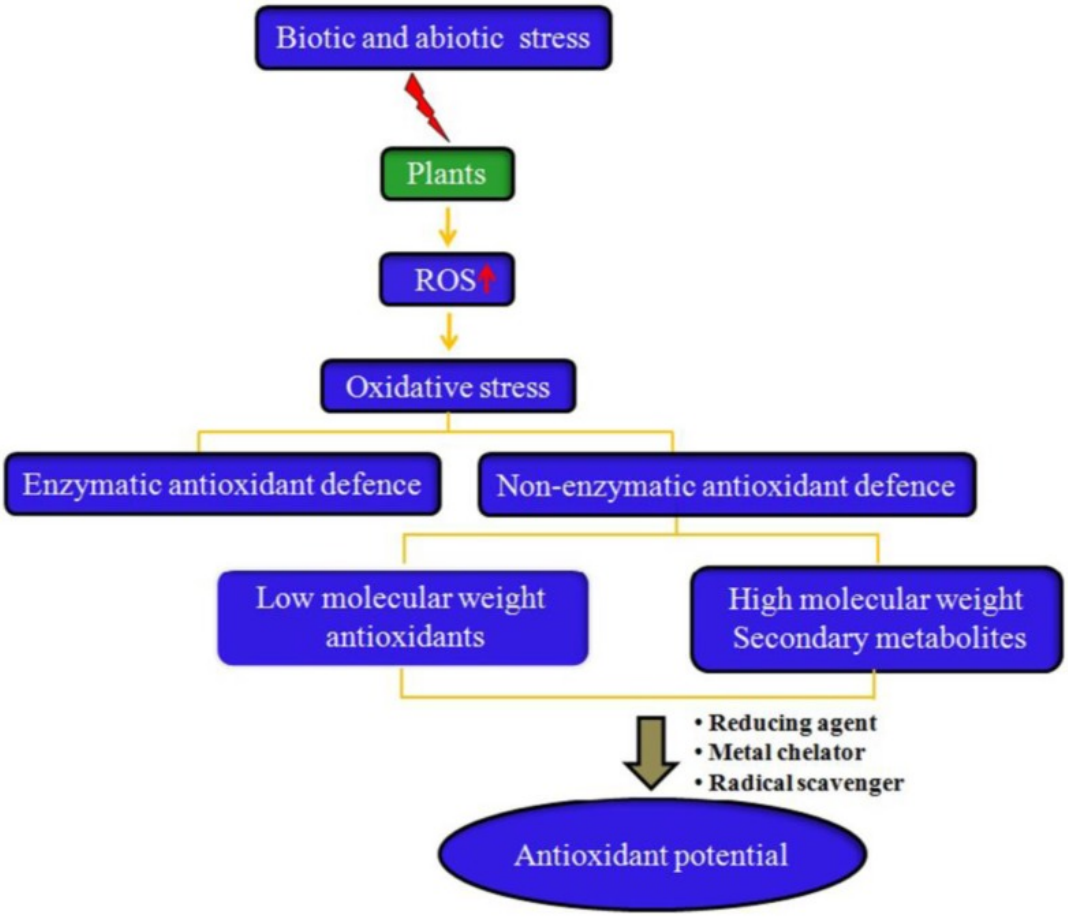


Figure: 1.2 Reasons behind all plants having antioxidant potentials (Kasote, Katyare, Hegde, & Bae, 2015).

Plants have a fundamental capacity to integrate non-enzymatic antioxidants. Nonetheless, under abiotic and also biotic stress conditions, the establishments of receptive oxygen species (ROS) augmentations in the plants are carrying acceptance of further oxidative pressure. For this prolonged oxidative pressure, plants increase the generation, collection of some cancer preventive agents having both low sub-atomic weight (ex: vitamin E, vitamin C, phenolic acids etc.) and high sub-atomic weight antioxidant metabolites which are

auxiliary. Tannins are example of such agents which give cancer prevention substances to most of the plants under in vitro contemplates by occupying as reducing agent, metal chelators, also free radical scavengers(Kasote, Katyare, Hegde, & Bae, 2015).

1.11 Phytochemical screening of plant leaf and stem:

The Greek word ‘phyton’ refers to plant and the term ‘phytochemical’ is originated from Greek culture. Phytochemistry is mainly the study related with plants substances. This study utilizes medications which are originated from plants parts to treat and eradicate various diseases(Kurmukov, 2013). It also can be defined as science based medical practice which is notable from other traditional practices. The medicinal plants show their medicinal activities mainly because of the phytochemicals (Sehwag & Das, 2014).These are naturally found in plants and offer biological activities to human. These chemical constituents protect plants from damage and equip those with identical color and flavor. Gradually the practice of using chemical constituents derived from plants is greater than before(Kurmukov, 2013).

The most important constituents of the plants are alkaloids, flavonoids, phenolic compounds, saponins tannins, proteins and amino acid(Edeoga, Okwu, & Mbaebie, 2005). These isolated constituents exert various pharmacological actions such as antibacterial, antiviral, antioxidant, anti-inflammatory, antifungal, ant diarrheal and so on. Because of these wide range activities as well as due to environmental safety natural medicines are expanding its popularity over synthetic medicines in recent years for disease management(Siddique et al., 2016). The phytochemicals present in fruits and vegetables play vital role to provide protective effect against colon, breast, lung and prostate cancer. Most of the cases, cancers occur due to the interaction of oxidant or carcinogens with DNA (Siddique et al., 2016).[i]The stringent antioxidant activity of the phytochemicals damages the oxidants and thus works against cancer. We know that phase I enzyme initiates carcinogenesis whereas phase II detoxify carcinogens. Phytochemicals inhibit the phase I enzymes and thus protect the DNA by inhibiting carcinogenesis(Omidiran et al., 2012).Moreover, phytochemicals inhibit the synthesis of LDL (Low Density Lipoprotein) or bad cholesterol and provide relief from cardio-vascular diseases. Some phytochemicals also

have anti-inflammatory activities. When Inflammation occurs there is a chance of platelets aggregation which causes blood clotting and ultimately stroke (Pryor, 2004). Learning of the chemical constituents of plants is attractive in light of the fact that such data will be of a motivation for the combination of complex synthetic ingredients.

1.12 Antidiarrheal activity of medicinal plants:

Due to unhygienic livelihood condition, peoples of the third world countries are very prone to several common diseases including diarrhea. According to (WHO), diarrhea is considered to be the second primary reason of death of kids less than 5 years of age (WHO, 2009). During diarrhea, the normal bowel movement becomes changed, which results in arise of water content, its volume or occurrence of the stools frequently (Guerrant et al., 2001).The common purpose for causing diarrhea is infection in the gastrointestinal tract by various types of pathogens, bacteria, parasites and virus. This contagion can be spread out through food, drinking water, and unhealthy environment. Besides other pathological conditions, usually four key mechanisms are accountable for pathophysiology in water and electrolytes transportation, such as increasing of osmolarity of lumen and secretion of electrolytes, decreasing of absorption of electrolytes and acceleration of motility of intestines which ultimately decrease transition time (Lutterodt, 1992). Regardless of the activities of international organizations to manage this illness, the frequency is still high (Mabeku Laure B. et al., 2007). A few antibiotics are utilized as antidiarrheal medicines, yet these medications in some cases demonstrate some unfriendly impacts and microorganisms are have a tendency to create opposition towards them(Mabeku Laure B. et al., 2007). In this manner the look for more efficient agents from plant beginning has kept on being a vital region of dynamic research. Be that as it may, plants have for quite some time been an imperative origin of new medications. Numerous plants have been selected for containing substances with helpful action (Maïkere-Faniyo, 1989). Additionally, numerous international organizations including WHO have urged consider relating to the treatment and counteractive action of diarrheal sicknesses utilizing conventional restorative practices (Lutterodt, 1989).In developing nations, diarrhea caused by infectious agent is a significant reason for baby mortality. The most elevated death rates have been accounted for the kids

under or 2 years old, with a death rate of 20 deaths for every 1000 population (Saha & Paul, 2012).

1.13 Toxicological activity of medicinal plants*:*

Medicinal plants should have protective effects yet several hazardous and lethal symptoms have been accounted for (Whitton, Lau, Salisbury, Whitehouse, & Evans, 2003). Natural or herbal medicines are generally considered as less harmful as they are natural substances(Gesler, 1992).Still, those contain bioactive compounds which cause adverse impacts (Bent, 2004). An adverse impact is defined as abnormal, undesirable or harmful changes after being exposed to any kind of toxic substances. The adverse effects may affect to the extent that it can lead to even death (Duffus, 2006). That's why, all the "natural" products having therapeutic potentials need to be submitted to efficacy and safety test (Talalay & Talalay, 2001).

Toxicology is the science which inspects the toxic substances. A toxin generally interacts with living cells and this is the crucial reason for causing serious damage to health. However, all substances have some poisonous effect and this effect can lead to death after excessive use. This lethal effect can be avoided if these chemicals can be used within tolerable limits (Duffus, 2006). The suitability of dose of a drug should be measured by previous studies of acute toxicity. Such studies are important to put off any overdose of drug which may obstruct the normal therapeutic effects and demonstrate lethal effects. For this project the acute and sub-acute toxicity tests were performed.

Acute toxicity:

This test is performed for the confirmation of absence of any substances that can cause acute toxicity to the users. It measures the possible short-term adverse effects. A particular amount of extract is injected into the vein or abdominal cavity of the mice. The mice are observed for any clinical change which is done by visual inspection. The test mainly focuses on the changes from their normal state or any lethal effects which eventually confirms the existence of any toxic substances in the extract (Duffus & Worth, 2013)

Sub-acute toxicity:

Sub-acute toxicity studies assess the toxic potentials and pathological properties of a drug for a period of 28 days. Throughout the study, data about mortality, physical examination, body weight, observed effects, hematology, bone marrow, food/water consumption, organ weights, coagulation, blood chemistry, urinalysis, histopathology, bio-chemistry, gross pathology, are mainly analyzed(Olaniyan, Muhammad, Makun, Busari, & Abdullah, 2016).

Chapter 2

Methodology

2. Methodology:

2.1 Collection and identification of the plant sample

For this project *Actephila excelsa* was chosen as no previous study was conducted on the plant's antioxidant activity, antidiarrheal activity, phytochemical screening and toxicological activity. The plant part both stems and leaves were used for this investigation. The plant was collected in January 2018 from Sylhet, Bangladesh. Then, for the authentication purpose, it was given in to the National Herbarium of Bangladesh (NHB), Mirpur. After that the plant identification accession number (42928) was collected.

Scientific name of the plant	Family	Parts used
Actephila excelsa	Phyllanthaceae	Leaf, stem

2.2 Extraction procedure:

The whole extraction procedure was followed into two steps:

a) Preparation of the plant material
b) Extraction procedure

2.3 Procedure of preparing the plant material:

Drying of the plant material:

At first the plant material was collected, washed and dried out properly using the shed drying process for the removal of water content and facilitate the drying process. It took about 1 month to completely remove the moisture from the plant.

Preparation of powdered plant material:

After the complete drying of the plant, the grinding of the plant was completed properly. To ensure the absence of water, the plants particles were inspected visually. The grinding was performed until complete powder formation.

2.4 Extraction and filtration of the powdered plant material:

For the extraction process, about 750 gm of powdered material of both plant and stem were taken and dissolved into methanol in amber bottle jars in order to facilitate the dissolution of the materials. The jars were kept in this manner for 1 week and were stirred on regular basis. The whole mixture was filtered using triple filtration method for the purification purpose.

- Firstly, through cloth
- Secondly, through cotton
- Thirdly, through Whatnam filter paper (extra-large size)

2.5 Evaporation of the solvent and collection of crude plant extract:

After completing the filtration process, the dissolved plant material was taken to the rotary evaporator (Heidolph Rota-vap 1500 rotary evaporator, Heidolph Laboratory Equipment, Germany) and the process was continued for 4 hours separately for stem and leaf. The temperature was set at 50°c to 60°c and the evaporator was set at speeds between 90 to 100 rpm. Then, the plant material was taken in Petri dishes and covered with aluminum foil having small pores for the exposure to air. This was done for further 7 days and the resultant extract weighed about 50gm for stem and 50gm for leaf.

2.6 Antioxidant evaluation of plant extract by DPPH frees radical scavenging:

It is an in vitro method which is used for the determination of the free radical scavenging property of the natural plant with the aid of spectrophotometer. DPPH (2,2-diphenyl-1-picrylhydrazyl) is widely accepted and used to determine the capability of compounds to perform as scavenger and also the antioxidant potential of plant extracts. The advantages of this are as follows:

- It can calculate the antioxidant potential of the sample quantitatively
- It can be utilized for both solid and liquid samples.

Preparation of the sample solution:

2mg of sample was dissolved in 4ml of methanol in a vial to make 500µg/ml concentration. Then it was serially diluted to 0.977µg/ml by taking 2ml from previous vial and adding 2 ml methanol to it.

Preparation of the standard solution:

2mg of Ascorbic acid was dissolved in 4ml of methanol in a vial to make 500µg/ml concentration. Then it was serially diluted to 0.977µg/ml by taking 2ml from previous vial and adding 2 ml methanol to it.

Preparation of DPPH solution

5mg of DPPH (2, 2-diphenyl-1-picrylhydrazyl) was added with 250ml methanol. So, the concentration was 20µg/ml.

Blank solution:

For blank, methanol was used.

Assay of DPPH free radical scavenging activity:

3ml DPPH solution was added to each of the vials containing the sample solution and also the standard solution. Then, this solution was kept in rest for 30 minutes in any dark place to let reaction be occurred among them. Afterward, the absorbance was measured at 517nm wavelength by UV spectrometer.

Calculation:

Percentage of inhibition was denoted by (I %):

$(I\ \%) = (1 - A_{sample}/A_{blank}) \times 100$

Where,

A_{blank} = absorbance of blank or control solution (contains all of the reagents excluding assessed one)

A_{sample}= absorbance of the sample solution

At last, (IC50) or the 50% inhibition concentration was obtained by plotting percentage of inhibition

(I %) against the extract concentration (μg/ml) in the graph.

2.7 Determination of (TPC) Total Phenolic content:

Preparation of test sample for evaluation:

To make 2mg/ml sample concentration, 2mg of extract was dissolved in 1ml distilled water.

Gallic acid solution preparation for standard curve:

For standard, Gallic acid is globally used for total phenolic content determination. For this test, various concentrations of Gallic acid solution were made ranges from 100μg/mL to 0μg/ml. The solution was prepared by mixing 2.5 mL of Folin-Ciocalteu chemical, 2mL of (7.5% w/v) sodium carbonate solution and 0.5 mL of Gallic acid. Folin-Ciocalteu chemical was diluted 10 times with water. The mixture was then kept in dark place for 20 minutes. After 20 minutes, absorbance was taken at 760nm by using UV spectrometer. A linear equation was obtained by plotting sample concentration against absorbance.

Preparation of the blank:

For the blank solution, 2.5 ml of FCR, 2ml of sodium carbonate and 0.5 ml of methanol was used.

Assay of Total phenolic content test:

Half mL of sample (2 mg / mL), 2.5 mL of 10 times diluted Folin-Ciocalteu and 2.0 mL of (7.5% w/ v) Na2CO3 were mixed together. The mixture was then kept in dark for 20 minutes. After passing the required time absorbance was noted with UV spectrophotometer at 760nm. With the analysis of standard curve of Gallic acid estimation was made for the total sample. Sample's Total phenolic content was affirmed as mg of GAE (Gallic acid equivalent)/g of extract.

2.8 Determination of total flavonoid content:

Reagent preparation:

At first, 10 gm of Aluminium chloride was measured and taken in a volumetric flask. It was dissolved in water up to 100 mL to make 10% aluminium chloride. Then, 9.815 gm of Potassium acetate was measured and taken in a volumetric flask of 100mL. It was dissolved in distilled water up to 100 mL to prepare 1M potassium acetate.

Sample and standard preparation:

For the sample, one twenty mg of the extract was dissolved in methanol of 10 ml. The concentration became 12mg/ml. Then three serially diluted concentrations of 1200, 800, 400µg/mL were made. Here, Quercetin was used as standard. The diluted concentrations of standard (1200, 800, 400 µg/mL) were made by the same manner.

Blank preparation:

For preparing the blank solution, 200 µL of 10 % $AlCl_3$solution, 200 µL of 1M Potassium acetate solution, 5.6 mL of distilled H_2O and 4 mL of methanol were used.

Experimental procedure:

At first, 1 mL of both of sample extract and standard (Quercetin) were taken in separate test tubes. In the test tubes 3mL of methanol was mixed. 200 µL of Aluminium chloride and Potassium acetate were also added to both of the test tubes. Followed by this, 5.6 mL of distilled water was also added to that. Both the tubes were kept for 30 minutes. Afterwards, the absorbance was measured at 415 nm by using spectrophotometer against blank. Lastly, the total flavonoid content C, of sample was expressed as Quercetin Equivalents (QE) using the following equation:

$C = (c \times V)/m$

Where,

C stands for Total content of flavonoid, mg of quercetin per gram of extract; generally it is expressed as (QE)

c = Quercetin concentration (µg/mL)

V = Volume of sample solution (mL)

m = weight of the sample (g)

2.9 Phytochemical screening of plant leaf and stem:

Table: 2.1. Reagent used for different chemical group test

Chemical groups	Reagents	Test
Carbohydrates	Molisch Reagent Fehling's Reagent	Molisch Test Fehling Test
Flavonoids	Zinc Ribbon and Concentrated Hydrochloric acid Sodium Hydroxide and Dilute Hydrochloric Acid	Flavonoids test
Proteins	10% Sodium Hydroxide 3% Copper Sulphate	Biuret's test
Saponins	Water	Frothing Test
Tannins	10% Potassium Dichromate 5% Ferric Chloride 1% Lead Acetate	Potassium Dichromate Test Ferric Chloride Test Lead Acetate Test
Alkaloids	Mayer's Reagent Dragendroff's Reagent Hager's Test	Mayer's Test Dragendroff's Test Hager's Test
Steroids	Sulphuric acid	Salkowski Test

Throughout this evaluation, the major therapeutic compounds of the plant extract can be found out. For this purpose, with few drops of Di-methyl sulphoxide, 40mg of the extract was dissolved in 40ml of water. In case of alcoholic solution, 10mg of extract was dissolved with 10ml of methanol. The process was carried out according to the description by (Ghani, 2005).

2.9.1 Preparation of reagent used:

The reagents were made accordance to the procedure prescribed by (Ghani, 2005)

Mayer's Reagent: 1.36gm of Mercuric Iodide was taken in a test tube and 60 ml of water was added with it. This was added with a solution of 5gm of KI and about 20ml of distilled H_2O. Finally the volume was 100ml.

Dragendroff's Reagent: 1.7gm of bismuth nitrate with 20gm of tartaric acid was taken in a test tube containing 80ml of distilled water. This was then mixed with a solution of 16gm of potassium iodide and 40ml of water. Before use, 10% dilution is made with picric acid

Hager's solution: 1gm of picric acid was added with 100ml of distilled water.

Fehling's solution A: 6.93gm of copper sulphate was mixed in a mixture that contains 0.1ml of conc. sulphuric acid and distilled water to make the volume 100ml .

Fehling's solution B: 35.2 gm of sodium potassium Tartarate was added with 15.4 gm of sodium hydroxide and made the final volume 100ml with distilled water.

Molisch solution: Around 2.5gm of α-napthol was mixed in 25ml ethanol.

10% Potassium Dichromate solution: 10gm of Potassium Dichromate and 100ml of distilled water was mixed**.**

5%Ferric Chloride solution: To prepare this solution 5gm of Ferric Chloride was dissolved with 100ml of distilled water.

1% Lead Acetate solution: To prepare this solution 1gm of Lead Acetate was dissolved with 100ml of distilled water.

10% Sodium Hydroxide solution: To prepare this solution 10gm of sodium hydroxide was dissolved in 100ml of distilled water.

3% Copper Sulphate solution: To prepare this solution 3gm of copper sulphate was added to 100ml of distilled.

Procedures of screening of phytochemicals:

The screening was done by visual inspection of color changes by the process described by (Ghani, 2003)

Test for Carbohydrates:

a. **Molisch Test**:

2ml extract solution was added with 2 drops of 10% alcoholic solution of alpha napthal in a test tube. Then, sulphuric acid was added inclined to the tube. If acid forms a red or reddish violet ring it would confirm the presence of carbohydrate. After standing, the solution would be dark purple.

b. **Fehling's Test (Standard Test for Reducing Sugars)**

1ml of Fehling's solution A and B was mixed with 2ml aqueous solution of extract and boiled it. If red or brick-red precipitate would be formed it would assure the presence of carbohydrate.

Tests for Tannins :

a. **Ferric Chloride Test**

5ml aqueous extract solution was taken in with 1ml of 5% Ferric solution in a test tube. Greenish black precipitation would confirm that tannins were present.

b. **Potassium dichromate Test**

1ml of 10% Potassium Dichromate was mixed with 5ml aqueous extract solution in a test tube. Yellow precipitate would confirm the presence of tannins.

c. **Lead acetate Test**

1%solution of lead acetate with 5ml aqueous extract solution was mixed. Yellow or red precipitate confirms that tannins were present.

Test for Flavonoids:

0.5ml of alcoholic extract of the sample was taken in a test tube and small piece of zinc dust and 0.5ml of alcoholic extract was mixed and boiled for few minutes. Emergrence of red to crimson color would confirm that flavonoid is present.

Test for Saponins:

Frothing Test

0.5ml of alcoholic extract was diluted with distilled water up to 10ml. It was shaken for 3-5 minutes. Continuous presence of frothing would confirm the positive test.

Test for Proteins

1ml of aqueous extract, 5-6 drops of 10% sodium hydroxide and 1-2 drops of 3% copper sulphate were mixed. Presence of red or violet colour would confirm the existence of proteins.

Test of Steroids:

Salkowski Test

2ml chloroform sample solution and 1ml of sulphuric acid was added together. The red color would confirm steroids exist.

Mayer's Test

2ml aqueous extract solution and 0.2 ml of conc. HCl were mixed with 1ml of Mayer's reagent. Presence of yellow precipitation would prove that alkaloids exist.

b. **Dragendroff's Test**

2ml aqueous extract solution and 0.2 ml of conc. HCl were mixed with 1ml of Dragendroff's reagent. Presence of orange brown precipitation would prove that alkaloids exist.

c. **Hager's Test**

2ml aqueous extract solution and 0.2 ml of conc. HCl were mixed with 1ml of Hager's reagent. Presence of yellow crystalline precipitation would prove that alkaloids exist.

2.10 Evaluation of antidiarrheal activity of *Actephila excelsa:*

For this experiment, castor oil and $MgSO_4$ induced method of diarrhea was followed to estimate the antidiarrheal activity of the extract of leaf and stem of *Actephila excelsa*. For this, each rat was given 1mL of both castor oil and $MgSO_4$ separately to induce diarrhea. The counts of feces were noted for each mouse after particular time interval. The percentages of inhibitions of extracts were compared with the standard group to determine the antidiarrheal activity.

Experimental animal:

For experimental purpose, the rats were purchased from International Centre for Diarrheal Diseases and Research, Bangladesh (ICDDR, B). These were kept in appropriate environmental state in a temperature of 21±1°C and fed properly for 3-4 days to adjust with the environment.

Experiment design:

Twenty four rats were selected and allocated them into four different groups each consisting of six rats for both the extracts of leaf and stem. First group was treated with control, 2^{nd} group was treated with standard and other two groups (3^{rd} and 4^{th}) were treated with methanolic extracts of plants in doses of 200 and 400 mg/kg body weight. All the rats were weighed with accuracy.

Preparation of Test Materials:

To treat the rats with extract, at first the extracts were dissolved in 0.8 mL of distilled water. To treat the rats with standard, standard was dissolved in 100 ml of distilled water where standard was loperamide HCL. To treat with the rats with control, 1mL distilled water was used.

Procedure:

All the rats of group-control were administered with 0.2 mL/kg distilled water orally. For the rats of standard group, they were orally administered with loperamide HCl (2mg/kg) into

control, positive control and test group consisting of six rats in each group. The sample groups were orally administered with methanolic extracts of leaf and stem of *Actephila excelsa* at 200 and 400 mg/kg body weight. After 1 hour, all the rats were treated with 1mL of castor oil and $MgSO_4$ separately. Rats were kept in cases individually keeping absorptive papers under the cases which were changed after 1 hour. This was continued for 4 hours after the administration of castor oil or $MgSO_4$ to observe the effect of diarrhea. The number of feces in the adsorptive paper was noted down at each successive hour for each rat. The percentages of inhibition of samples were calculated and then compared with the standard.

2.11 Toxicological studies:

Acute toxicology:

For the oral acute toxicity, the rats were divided into two groups where each group contains 5 rats. The first group was control group which was administered with normal saline water and the second group was administered with 1000mg/kg extract. For this purpose, the rats were kept fasted overnight before conducting the tests and their body weight was measured. The rats were observed for any symptoms or signs of toxicity or mortality for first four hours after the oral administration and thus for 14 days. Here the following parameters are checked which are digestion, body weight, temperature, food intake, urination, rate of respiration, change in skin, drowsiness, sedation, eye color, diarrhea, death.

Sub-acute toxicology:

In sub-acute toxicity, the rats were divided into three groups for both leaf and stem. The rats of control group were treated with saline water whereas the rats of second and third groups were treated with the extracts at 250mg/kg and 500mg/kg oral dose respectively at two days interval and thus for 28 days. Prior to administration of the extracts and saline, the weights of all the rats were taken and according to their weight the doses were calculated. The body weight was measured at day 1 and then after two days interval during the period of 28 days study. Then, the average body weight and organ (heart, liver, kidney, pancreas, spleen, lungs) weight was calculated using electric balance. The body and organ weights were then compared with the control group.

Statistical analysis:

For the statistical analysis, students' t test was performed and the p value was determined. If the p value was <0.005 then the difference was counted as significant and vice versa.

Hematological and biochemical examination:

At the end of 28th day, all the rats were fasted overnight and the next morning the rats were treated with chloroform for anesthesia and from the heart blood samples were taken by direct puncture. The collected blood samples were then placed in tubes of which half contained EDTA (anticoagulant) and other half was free from EDTA for hematological analysis. Blood samples which were free from EDTA were used for biochemical analysis and EDTA containing blood samples were used for hematological analysis. The blood samples containing no EDTA were centrifuged for 15 minutes at 2500rpm to get the serum which was stored at -20°C. For histopathological analysis, the liver and kidneys were collected in jars containing formalin.

Effect of plant extract on hematological parameters:

For this experiment, automatic hematology analyzer (Sysmex,Japan) was used. Hemoglobin, erythrocyte sedimentation rate, white blood cell, red blood cell, platelets count, different leucocyte (neutrophil, lymphocyte, eosinophil, basophil, mean corpuscular volume, mean corpuscular hemoglobin concentration, mean corpuscular hemoglobin, RBC distribution width, Platelet Distribution Width, Mean platelets volume of the control and samples of stem and leaf were determined and compared.

Effect of plant extract on serum biochemical parameters:

The biochemical analysis was performed using clinical chemistry analyzer (Vital Scientific, Netherlands). Here, serum was used and the following parameters were checked:
random blood sugar, creatinine, urea, bilirubin, alkaline phosphatase(ALP) , alanine transaminase (ALT), aspartate transaminase (AST), high density lipoprotein, low density lipoprotein, triglycerides, total cholesterol and electrolytes (albumin, globulin, protein, uric acid, calcium, sodium, potassium, chloride) for both control and test samples of stem and leaf.

Chapter 3

Result

3.1 Antioxidant property analysis:

3.1.1 Evaluation of DPPH free radical scavenging assay of leaf and stem of *Actephila excelsa*

Table 3.1 Determination of IC_{50} value of Ascorbic acid (ASA)

Concentration (µg/ml)	Absorbance of Standard (ASA)	% of inhibition	IC_{50} µg/mL
500	0.031	94.97568882	75.688
250	0.043	93.03079417	
125	0.065	89.46515397	
62.5	0.184	70.17828201	
31.25	0.292	52.67423015	
15.625	0.385	37.6012966	
7.813	0.418	32.2528363	
3.906	0.464	24.79740681	
1.953	0.481	22.0421394	
0.977	0.497	19.4489465	
Blank	0.617		

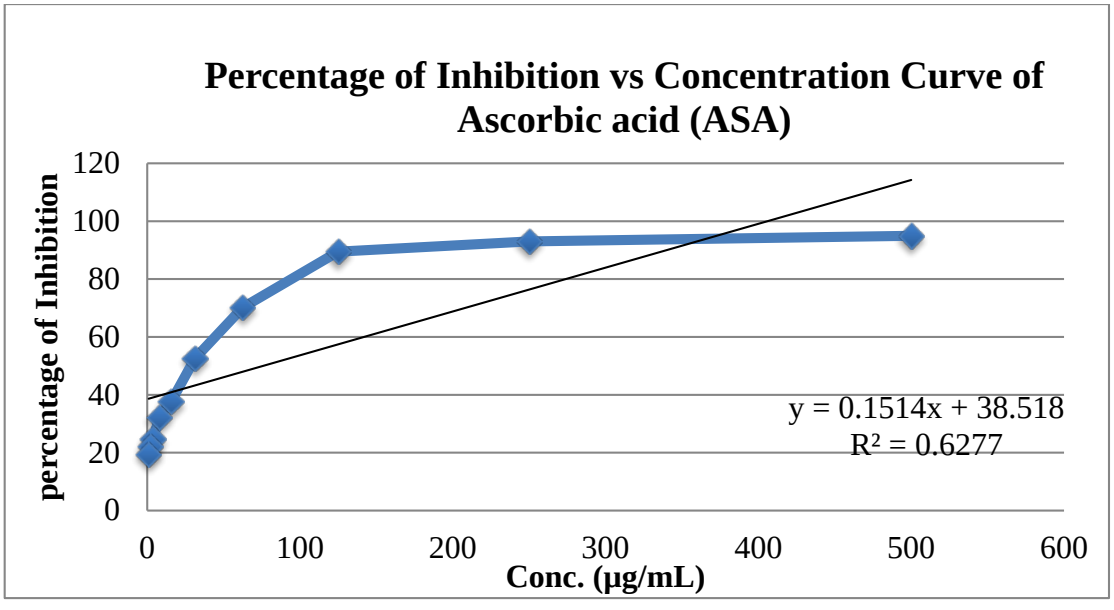


Figure 3.1 % Inhibition vs. Concentration curve of ASA

Table 3.2 IC_{50} value (µg/mL) of leaf of methanol extract of *Actephila excelsa*

Conc. (µg/ml)	Absorbance of Leaf	% of inhibition	IC_{50} µg/mL
500	0.183	70.34036	92.929
250	0.22	64.3436	
125	0.236	61.75041	
62.5	0.255	58.67099	
31.25	0.296	52.02593	
15.625	0.316	48.78444	
7.813	0.344	44.24635	
3.906	0.368	40.35656	
1.953	0.402	34.846	
0.977	0.437	29.1734	
Blank	0.617		

From table 3.1 and 3.2, it can be assumed that the IC_{50} value is much higher in leaf extract than the standard (Ascorbic acid)

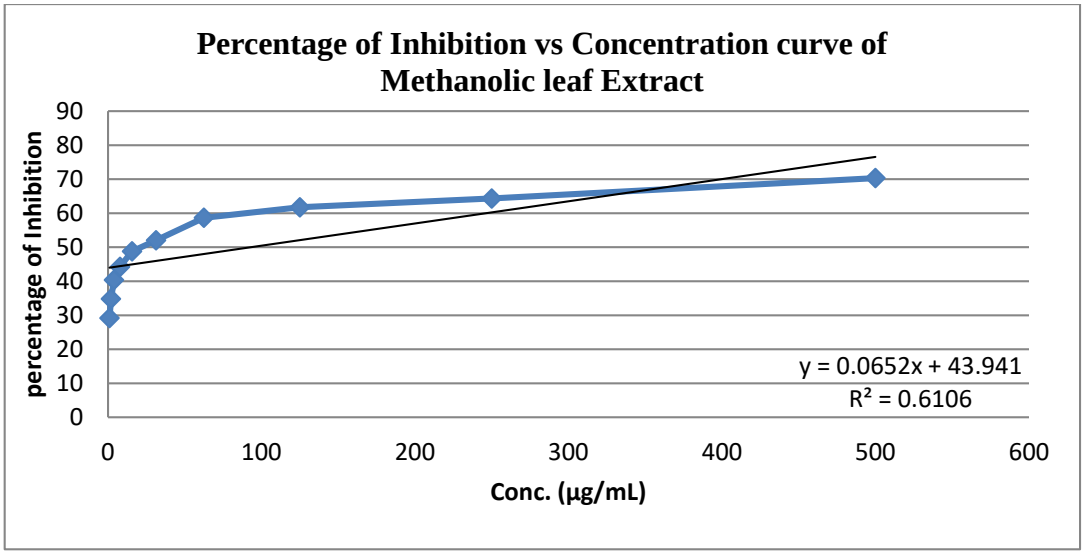


Figure 3.2 Percentage of Inhibition vs. Concentration curve of leaf of Methanol extract of *Actephila excelsa*

Table: 3.3 IC_{50} value (µg/mL) of stem of methanol extract of *Actephila excelsa:*

Conc. (µg/ml)	Absorbance of Stem	% of inhibition	IC_{50}µg/mL
500	0.051	91.7342	
250	0.052	91.57212	
125	0.085	86.22366	
62.5	0.182	70.50243	
31.25	0.324	47.48784	
15.625	0.431	30.14587	115.24
7.813	0.445	27.87682	
3.906	0.513	16.85575	
1.953	0.555	10.0486	
0.977	0.604	2.10697	
Blank	0.617		

By comparing table 3.1 and 3.3, it can be assumed that the (%) of inhibition is higher than the standard.

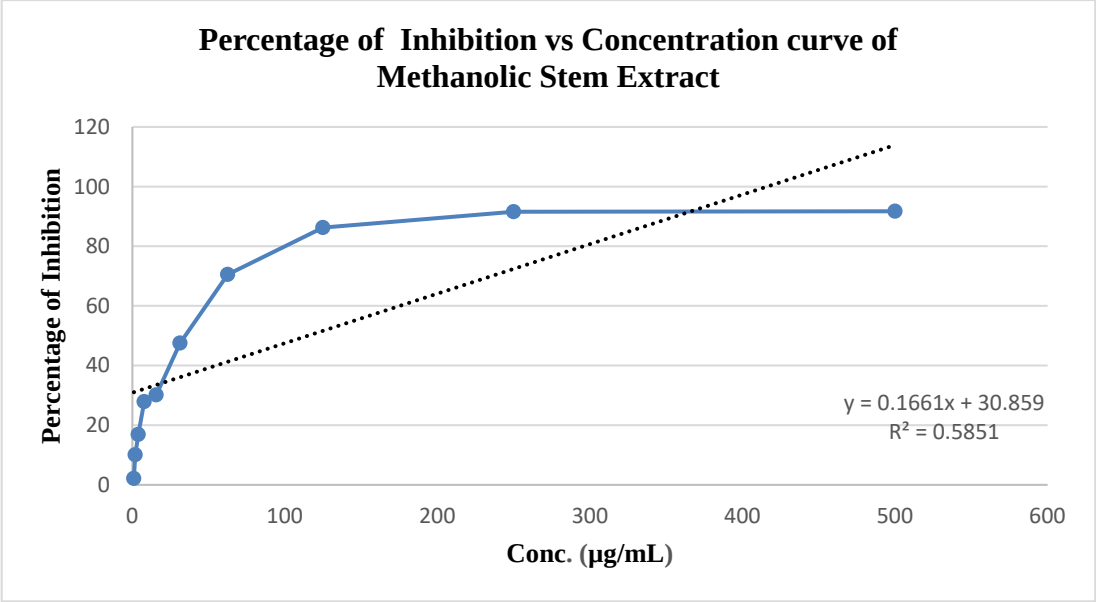


Figure 3.3 Percentage of Inhibition vs. Concentration curve of stem of Methanol extract of *Actephila excelsa*

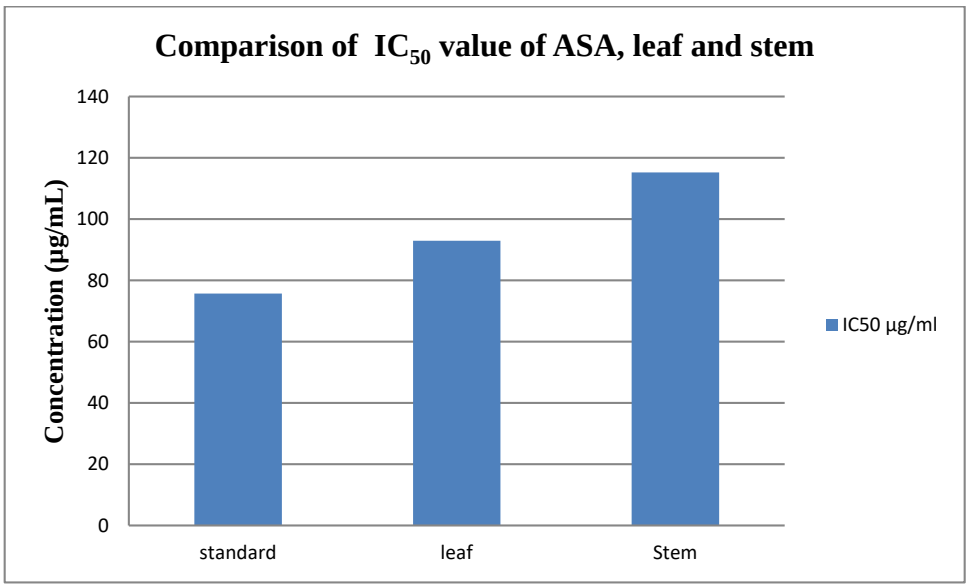


Figure 3.4 IC_{50} value of standard (ASA), leaf and stem of *Actephila excelsa*

The figure clearly illustrates that the IC_{50} value is higher in leaf and stem than the standard.

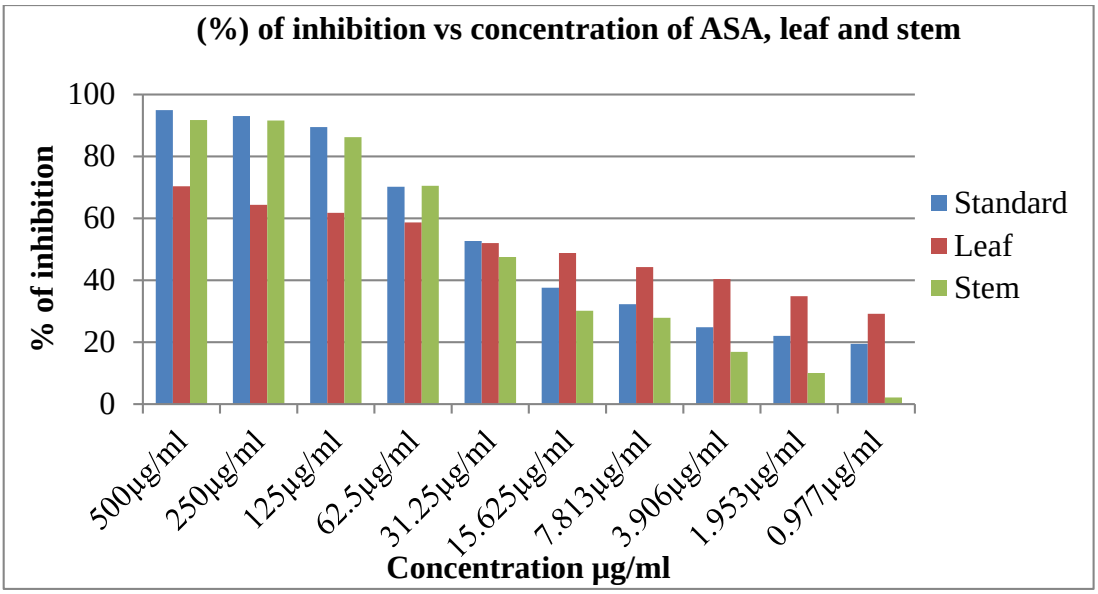


Figure 3.5: Percentage of inhibition of Free Radical Scavenging of leaf and stem of *Actephila excelsa* and Ascorbic Acid. It shows that leaf extract shows higher (%) of inhibition than standard and stem when the concentration is in descending order.

3.1.2 Determination of Total phenolic content:

Table 3.4 Absorbance of Gallic acid in different concentrations

Conc. (µg/ml)	**Absorbance**	**Regression line equation**	**R^2**
100	0.8	Y = 0.0081x-0.0007	0.9975
50	0.423		
25	0.215		
12.5	0.123		
6.25	0.047		
3.125	0.007		
1.5625	0.003		
0.78125	0		
0.3906	0		
0	0		

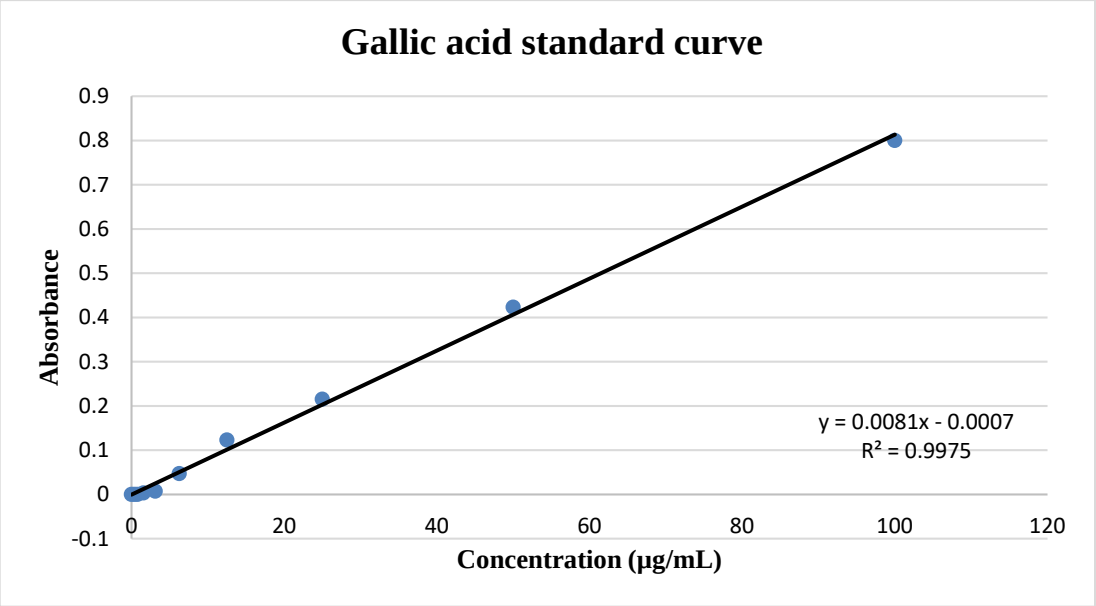


Figure 3.6 Standard curve for determination of Total Phenolic Content

Table 3.5 Total phenolic content of methanolic extract ofleaf of *Actephila excelsa*

Sample	**Absorbance**	**Total phenolic content (mg of GAE/gm of extract)**
Methanolic extract of leaf of *Actephila excelsa*	0.3093	382.75

Table 3.6 Total phenolic content of methanolic extract ofstem of *Actephila excelsa*

Sample	**Absorbance**	**Total phenolic content (mg of GAE/gm of extract)**
Methanolic extract of stem of *Actephila excelsa*	0.0761	94.8

Total phenolic contents of stem and leaf are 382.75 and 94.8 mg of GAE/gm of extract.

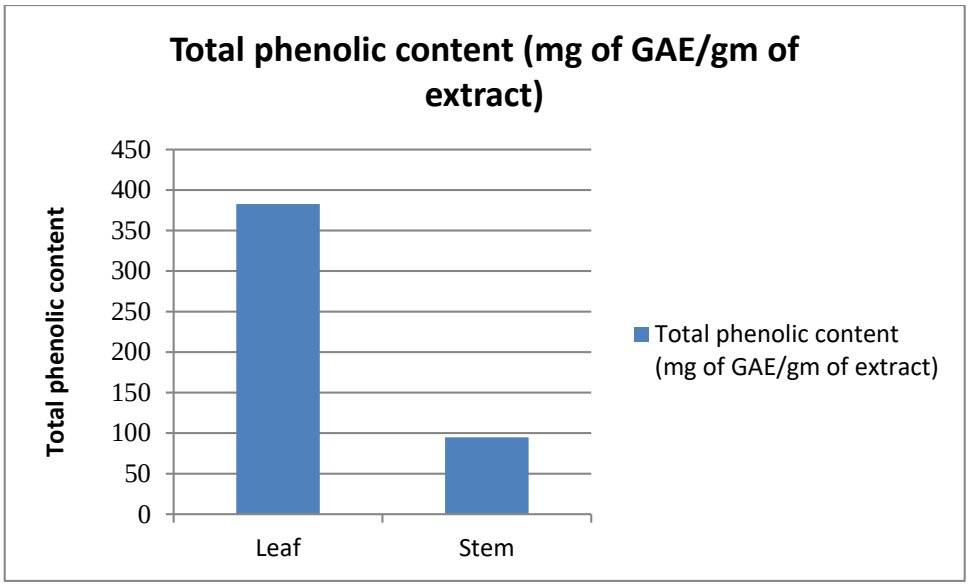


Figure 3.7 Total phenolic content of leaf and stem of *Actephila excelsa*

This figure shows that the leaf contains more antioxidant potentials than stem.

3.1.3 Determination of Total flavonoid content:

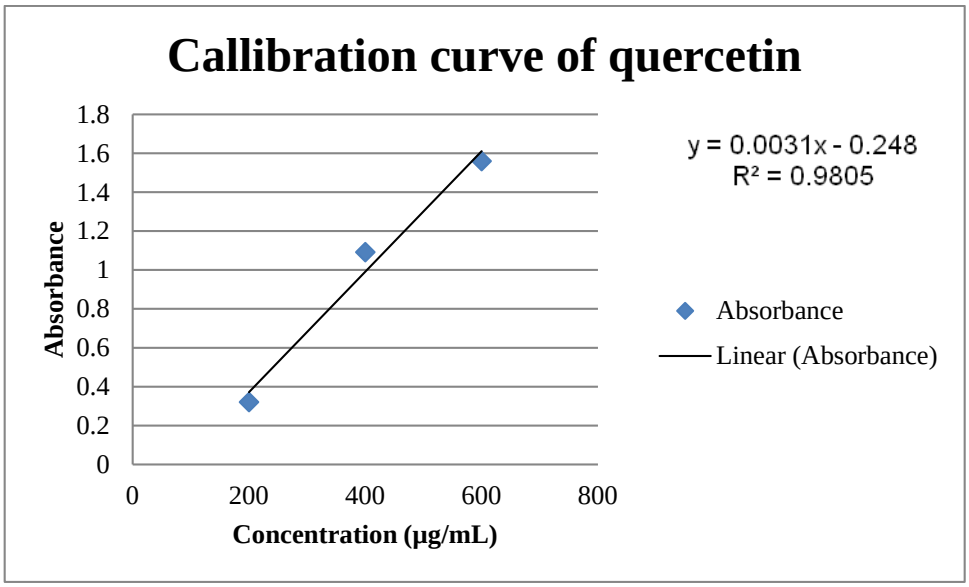


Figure 3.8: Standard Curve of Quercetin at 415 nm.

Table 3.7 Total flavonoid content (TFC) in stem of *Actephila excelsa*

Concentration stem of *actephila excelsa* (µg/mL)	**Absorbance values recorded at 415 nm**	**TFC(QE) mg of Quercetin per gram of dried extract**
200	0.430	118.83
400	0.298	190.69
800	0.132	247.83

Table 3.8 Total flavonoid content (TFC) in leaf of *Actephila excelsa*

Concentration stem of *actephila excelsa* (µg/mL)	**Absorbance values recorded at 415 nm**	**TFC(QE) mg of Quercetin per gram of dried extract**
200	0.392	104.11
400	0.215	154.76
800	0.098	231.38

With the increase of concentration the total flavonoid contents of methanolic extract of leaf and stemare also increased in significant amount.

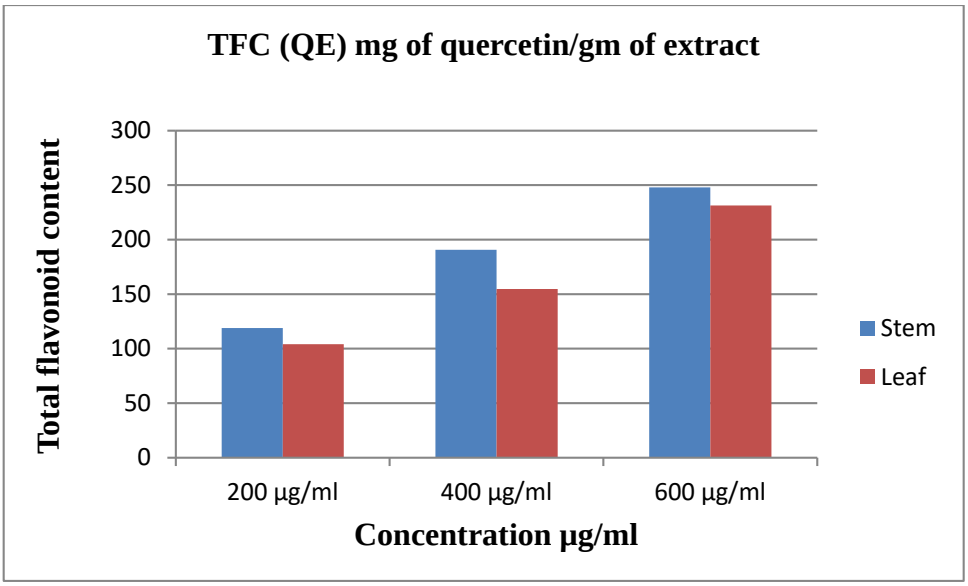


Figure 3.9 Total flavonoid content of stem and leaf of *actephila excelsa*

Figure shows that the stem contains higher total flavonoid content than the leaf and it is increasing with the increase of concentration.

3.2 Phytochemical screening of *Actephila excelsa*:

Table 3.9 Different tests of phytochemical screening of leaf and stem:

Chemical group	Test	Leaf	Stem
Carbohydrate	Molisch Test Fehling Test	(+) (-)	(+) (-)
Tannins	Potassium Dichromate Test Ferric Chloride Test Lead Acetate Test	(-) (+) (-)	(-) (-) (+)
Proteins	Biurets's test	(-)	(-)
Saponins	Frothing Test	(+)	(+)
Flavonoids	Flavonoid Test	(+)	(+)

Steroids	Salkowski Test	(-)	(-)
Alkaloids	Mayer's Test Dragendroff's Test Hager's Test	(+) (+) (+)	(+) (-) (-)

In the table the (+) indicates the presence and (-) indicates the absence of respective phyochemicals in the leaf and stem extract.

3.3 Evaluation of antidiarrheal activity of *Actephila excelsa:*

Here, all the rats were treated with 0.2ml/kg water for control, 2mg/kg loperamide HCl for standard and 200mg/kg, 400mg/kg leaf extract for sample. After 1 hour 1 ml of castor oil and$MgSO_4$ was administered respectively and no. of feces was counted at each hour.

Table 3.10 Data showing the total number of diarrheal feces given by each rat (leaf extract)

$MgSO_4$ induced diarrhea							
	number of mice	No of feces in 1st hour	No of feces in 2ndhour	No of feces in 3rd hour	No of feces in 4th hour	No of feces in total 4 hour	Average
Control (negative)	1	3	4	7	11	25	22.66667
	2	2	5	7	10	24	
	3	2	5	9	11	27	
	4	4	6	7	11	28	
	5	3	6	4	10	23	
	6	1	8	0	0	9	
Control (positive)	1	0	2	4	6	12	14
	2	0	4	5	7	16	
	3	0	3	5	7	15	
	4	0	4	4	7	15	

	5	0	3	4	8	15	
	6	0	2	4	5	11	
Sample 200mg/kg	1	2	4	6	9	21	20.5
	2	3	3	5	11	22	
	3	3	4	6	8	21	
	4	4	4	7	7	22	
	5	3	5	6	0	14	
	6	4	5	6	8	23	
Sample 400mg/kg	1	0	1	4	6	11	15
	2	0	3	5	6	14	
	3	0	4	5	7	16	
	4	0	3	6	8	17	
	5	0	3	4	8	15	
	6	0	4	5	8	17	

Table 3.11 Effect of methanolic extract of leaf on rats by the method of $MgSO_4$ induced diarrhea:

Treatment	**Standard deviation(SD)**	**Number of diarrheal feces (Mean ± SD)**	**% reduction of diarrhea**
Control (negative)	6.947422	22.66667 ± 6.947422	--------
Control(positive)	2	14 ± 2	38.24
Sample(200mg/Kg)	3.271085	20.5 ± 3.271085	9.55
Sample(400mg/Kg)	2.280351	15 ± 2.280351	33.82

(%) of reduction is higher in leaf sample (400mg/kg) than the standard.

Table 3.12 Data showing the total number of diarrheal feces given by each rat (leaf extract)

Castor oil induced diarrhea							
	number of mice	No. of feces in 1st hour	No. of feces in 2nd hour	No. of feces in 3rd hour	No of feces in 4th hour	Total no of feces for 4 hours	average
Control (negative)	1	2	4	6	9	21	20.5
	2	3	4	7	8	22	
	3	4	5	7	8	24	
	4	1	3	6	9	19	
	5	1	6	4	7	18	
	6	1	5	5	8	19	
Control (positive)	1	0	2	4	5	11	9.833333
	2	0	0	3	4	7	
	3	0	3	2	6	11	
	4	0	4	2	4	10	
	5	0	2	4	4	10	
	6	0	2	4	4	10	
Sample 200mg/kg	1	0	3	3	4	10	13.66667
	2	2	4	4	5	15	
	3	3	4	2	5	14	
	4	2	5	4	6	17	
	5	1	4	5	4	14	
	6	2	3	4	3	12	
Sample 400mg/kg	1	0	2	2	3	7	9.5
	2	0	1	3	4	8	
	3	0	1	3	4	8	
	4	0	1	4	3	8	
	5	0	2	4	5	11	
	6	0	3	5	7	15	

Table 3.13 Effect of methanolic extract of leaf on mice by the method of Castor oil induced diarrhea

Treatment	Standard deviation(SD)	Number of diarrheal feces (Mean ± SD)	% reduction of diarrhea
Control (negative)	2.258318	20.5 ± 2.258318	--------
Control(positive)	9.833333	9.833333 ± 1.47196	52.03
Sample(200mg/Kg)	13.66667	13.66667 ± 2.42212	33.33
Sample(400mg/Kg)	9.5	9.5 ± 3.016621	53.65

Table 3.13 shows that (%) of reduction is higher in leaf sample (400mg/kg) than the standard.

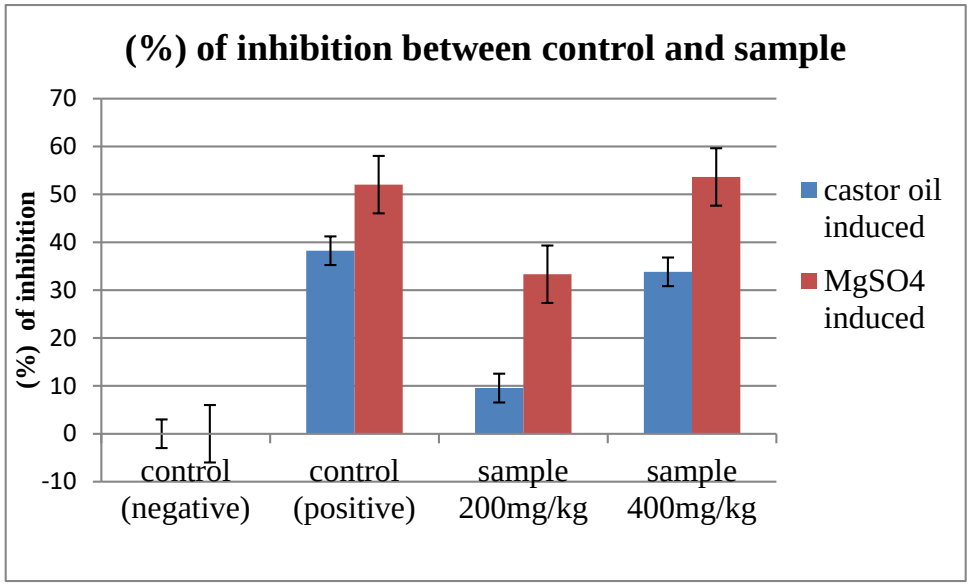


Figure 3.10 Comparison of Percentage of inhibition of standard and sample using castor oil and $MgSO_4$ method clearly indicates that the leaf extract has significant antidiarrheal activity that is higher than the standard.

3.4 Toxicological activity of stem and leaf of *Actephila excelsa*

For control group normal saline water was used and for samples 250mg/kg, 500mh/kg of both leaf and stem extracts were administered separately. Then the averages of body weights were calculated.

Table: 3.14 Different organ weights of rats of control group

Group name	**Control(negative)**							
Organ name	(i)	(ii)	(iii)	(iv)	(v)	Average	S.D.	Average± S.D.
Lungs	1.423	1.252	1.639	1.574	1.358	1.4492	0.157	1.4492± 0.157
Heart	0.46	0.513	0.493	0.498	0.49	0.4908	0.019	0.4908± 0.019
Liver	6.03	6.63	6.783	6.326	6.759	6.5056	0.322	6.5056± 0.322
Pancreas	0.615	0.668	0.814	0.964	0.846	0.7814	0.140	0.7814± 0.140
Spleen	0.634	0.79	0.897	0.758	0.82	0.7798	0.096	0.7798± 0.096
Kidneys	1.239	1.194	1.273	1.364	1.234	1.2608	0.064	1.2608± 0.064

Table: 3.15 Different organ weights of rats of leaf sample 250mg/kg

Group name	leaf sample at 250mg/kg dose							
Organ name	(i)	(ii)	(iii)	(iv)	(v)	Average	S.D.	Avg.± S.D.
Lungs	1.017	0.8	0.948	1.033	0.958	0.9512	0.092	0.9512±0.092
Heart	0.515	0.487	0.518	0.476	0.472	0.4936	0.021	0.4936± 0.021
Liver	6.094	4.877	5.807	5.556	5.604	5.5876	0.450	5.5876± 0.450
Pancreas	0.527	0.426	0.55	0.518	0.592	0.5226	0.061	0.5226± 0.061
Spleen	0.65	0.72	0.783	0.81	0.63	0.7186	0.079	0.7186± 0.079
Kidneys	0.842	0.732	0.824	0.811	0.8	0.8018	0.042	0.8018± 0.042

Table: 3.16 Different organ weights of rats of leaf sample 500mg/kg

Group name	leaf sample at 500mg/kg dose							
Organ name	(i)	(ii)	(iii)	(iv)	(v)	Average	S.D.	Avg.± S.D.
Lungs	0.787	0.876	0.667	0.85	1.041	0.8442	0.136	0.8442± 0.136
Heart	0.398	0.411	0.427	0.471	0.477	0.4368	0.035	0.4368± 0.035
Liver	4.078	4.93	3.444	4.76	5.136	4.4696	0.697	4.4696± 0.697
Pancreas	0.334	0.385	0.259	0.438	0.287	0.3406	0.072	0.3406± 0.072
Spleen	0.516	0.813	0.513	0.644	0.543	0.6058	0.127	0.6058± 0.127
Kidneys	0.604	0.807	0.617	0.833	0.828	0.7378	0.116	0.7378± 0.116

Table: 3.17 Different organ weights of rats of stem at 250mg/kg dose

Group name	Stem sample at 250mg/kg dose							
Organ name	(i)	(ii)	(iii)	(iv)	(v)	Average	S.D.	Avg. ± S.D.
Lungs	0.833	0.83	0.779	0.833	0.901	0.8352	0.043	0.8352± 0.043
Heart	0.387	0.45	0.383	0.425	0.447	0.4184	0.032	0.4184± 0.032
Liver	3.46	4.407	4.241	3.748	3.946	3.9604	0.378	3.9604± 0.378

Pancreas	0.265	0.286	0.294	0.343	0.358	0.3092	0.039	0.3092± 0.039
Spleen	0.447	0.576	0.594	0.566	0.698	0.5762	0.089	0.5762± 0.089
Kidneys	0.643	0.639	0.699	0.636	0.781	0.6796	0.0623	0.6796± 0.062

Table: 3.18 Different organ weights of rats of stem at 500mg/kg dose

Group name	Stem sample at 500mg/kg dose							
Organ name	(i)	(ii)	(iii)	(iv)	(v)	Average	S.D.	Avg. ± S.D.
Lungs	0.798	0.718	0.6	0.827	0.999	0.7884	0.146	0.7884 ±0.146
Heart	0.389	0.388	0.39	0.474	0.345	0.3972	0.046	0.3972 ± 0.046
Liver	4.203	3.37	3.274	4.574	3.842	3.8526	0.550	3.8526 ± 0.550
Pancreas	0.275	0.274	0.2	0.262	0.245	0.2512	0.031	0.2512 ± 0.031
Spleen	0.527	0.525	0.522	0.714	0.636	0.5848	0.086	0.5848 ± 0.086
Kidneys	0.685	0.647	0.616	0.85	0.644	0.6884	0.093	0.6884 ± 0.093

Table: 3.19 Effects of oral administration of methanolic leaf and stem extract of *Actephila excelsa* on organ weights of rats:

organ	Treatment groups				
	Control	250mg/kgbw (leaf)	500mg/kgbw (leaf)	250mg/kgbw (stem)	500mg/kgbw (stem)
Lungs	1.4492 ±0.157	0.9512 ± 0.092 *	0.8442 ± 0.136 *	0.8352 ± 0.043 *	0.7884 ± 0.146 *
Heart	0.4908 ±0.019	0.4936 ± 0.021	0.4368 ± 0.035 *	0.4184 ± 0.032 *	0.3972 ± 0.046 *
Liver	6.5056 ±0.322	5.5876 ± 0.450 *	4.4696 ± 0.697 *	3.9604 ± 0.378 *	3.8526 ± 0.550 *
Pancreas	0.7814 ±0.140	0.5226 ± 0.061 *	0.3406 ± 0.072 *	0.3092 ± 0.039 *	0.2512 ± 0.031 *
Spleen	0.7798 ±0.096	0.7186 ± 0.079	0.6058 ± 0.127 *	0.5762 ± 0.089 *	0.5848 ± 0.086 *
Kidneys	1.2608 ±0.064	0.801±0.042 *	0.737±0.116 *	0.679±0.062 *	0.688±0.093 *

Here, the average ± S.D organ weights of the rats were calculated and (*) mark is put on those values having p value (<0.05) which represents significant difference of the organ weights between control and experimented groups.

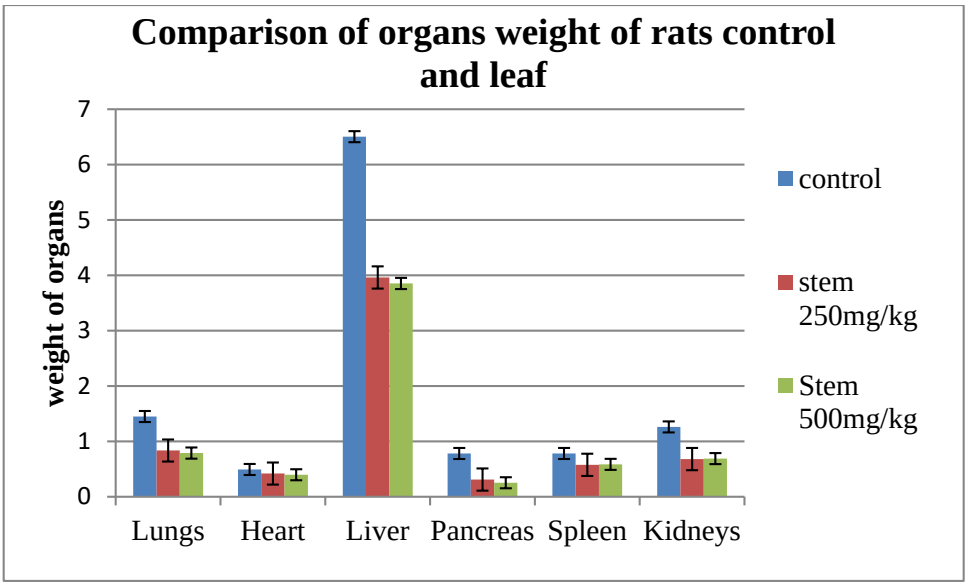


Figure 3.11 Comparison of organs weight of rats control and stem

Table: 3.20 Body weights of rats of control group

Group name	**Control(Negative)**						
Day	(i)	(ii)	(iii)	(iv)	(v)	Average	S.D.
1	90	90	105	105	130	104	16.35
3	94	95	110	114	105	103.6	8.905
5	96	100	115	116	110	107.4	8.988
7	100	105	120	120	113	111.6	8.961
9	105	109	125	122	115	115.2	8.438
11	110	115	127	125	119	119.2	7.014
13	115	120	129	127	125	123.2	5.674
15	120	125	133	128	129	127	4.847
17	125	130	135	133	131	130.8	3.768
19	130	133	140	136	134	134.6	3.714
21	135	137	145	140	138	139	3.807
23	138	140	147	144	143	142.4	3.507

26	140	142	150	146	145	144.6	3.847
28	143	145	153	148	148	147.4	3.781

Table: 3.21 Body weights of rats of leaf sample at 250mg/kg dose

Group name	leaf sample at 250mg/kg						
Day	(i)	(ii)	(iii)	(iv)	(v)	Average	S.D.
1	90	95	100	110	120	103	12.041
3	95	97	102	113	122	105.8	11.432
5	100	100	108	115	128	110.2	11.755
7	105	105	111	117	130	113.6	10.430
9	110	108	115	120	133	117.2	9.984
11	113	113	117	125	135	120.6	9.423
13	115	115	120	130	136	123.2	9.418
15	117	117	123	133	137	125.4	9.208
17	120	120	125	135	138	127.6	8.443
19	125	122	130	140	141	131.6	8.619
21	130	124	136	148	144	136.4	9.838
23	135	125	139	150	147	139.2	9.959
26	148	126	142	153	149	143.6	10.597
28	148	129	144	155	150	145.2	9.884

Table: 3.22 Body weights of rats of leaf sample at 500mg/kg dose

Group name	leaf sample at 500 mg/kg						
Day	(i)	(II)	(iii)	(IV)	(v)	AVG	S.D.
1	85	95	100	110	120	102	13.509

3	86	100	101	115	125	105.4	15.010
5	88	103	103	120	128	108.4	15.757
7	91	104	104	122	129	110	15.313
9	95	105	105	125	130	112	14.832
11	102	105	107	128	133	115	14.370
13	105	105	109	130	135	116.8	14.532
15	106	108	113	133	137	119.4	14.536
17	107	110	114	135	138	120.8	14.584
19	108	115	117	140	139	123.8	14.720
21	110	120	120	144	140	126.8	14.532
23	120	130	123	148	145	133.2	12.716
26	130	136	125	151	150	138.4	11.717
28	130	140	127	153	151	140.2	11.819

Table: 3.23 Body weights of rats of stem sample at 250mg/kg dose

Group name	Stem sample at 250mg/kg						
Day	(i)	(ii)	(iii)	(iv)	(v)	Average	S.D.
1	85	95	100	115	115	102	13.038
3	85	98	105	117	118	104.6	13.794
5	85	100	110	119	120	106.8	14.618
7	86	105	115	121	123	110	15.132
9	88	110	125	123	125	114.2	15.927
11	89	115	125	124	127	116	15.779
13	100	120	125	125	130	120	11.726
15	102	123	127	128	135	123	12.51
17	105	125	128	130	140	125.6	12.817
19	108	130	130	133	145	129.2	13.367

21	110	135	135	138	150	133.6	14.570
23	115	137	138	139	155	136.8	14.254
26	120	147	141	140	160	141.6	14.467
28	121	150	144	144	162	144.2	14.906

Table: 3.24 Body weights of rats of stem sample at 500mg/kg dose

Group name	Stem sample at 500mg/kg						
Day	(i)	(ii)	(iii)	(iv)	(v)	Average	S.D.
1	85	95	100	115	115	102	13.038
3	88	98	102	117	118	104.6	12.837
5	90	100	104	120	120	106.8	13.084
7	93	101	105	123	123	109	13.490
9	95	104	105	125	125	110.8	13.535
11	99	107	108	130	131	115	14.577
13	110	110	110	140	135	121	15.165
15	115	115	111	145	136	124.4	15.126
17	118	118	113	150	138	127.4	15.868
19	125	119	117	155	140	131.2	16.068
21	130	120	120	160	144	134.8	17.181
23	138	125	125	163	147	139.6	16.056
26	144	130	128	168	150	144	16.309
28	147	133	129	170	153	146.4	16.456

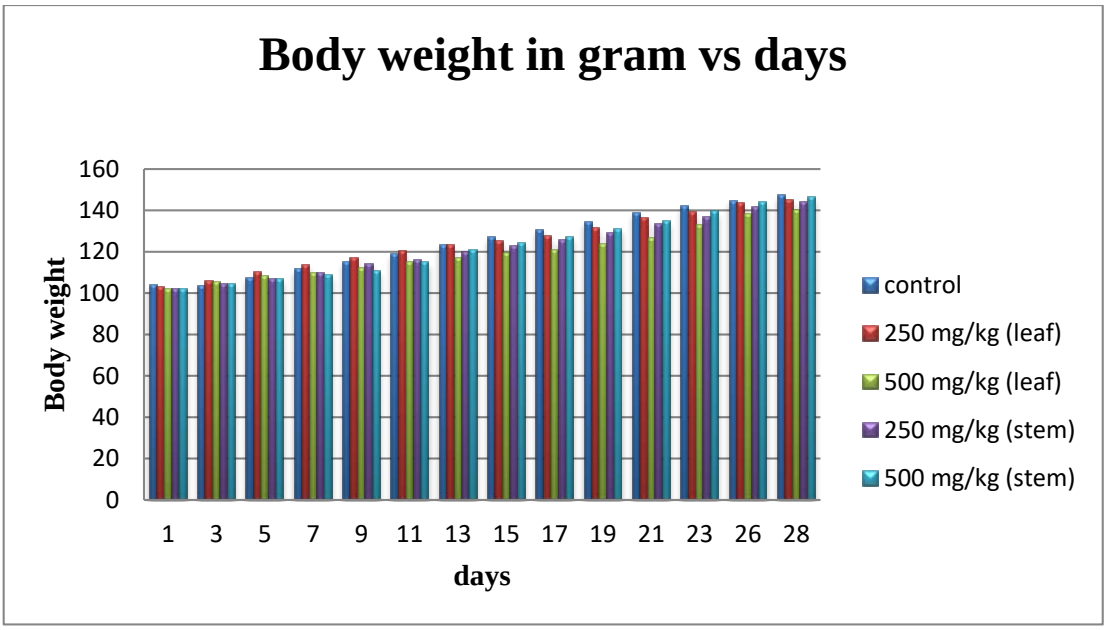


Figure: 3.12 Comparison of changes in body weight of rats of group(control, leaf, stem) with the increase of days.

In the figure, it is clearly visible that there are no significant changes in body weight of the rats in comparison to control. The body weights are increasing with the increase of days for both the control group and experimental group. Insignificant decrease in body weight is observed in the rats treated with 500mg/kg leaf extract.

Table: 3.25 Effects of oral administration of methanolic leaf and stem extract of *Actephila excelsa* on body weights of rats:

Group name	**Treatment group**				
Day	Control	250mg/kgbw (leaf)	500mg/kgbw (leaf)	250mg/kgbw (stem)	500mg/kgbw (stem)
1	104±16.355	103 ±12.041	102 ±13.509	102 ±13.038	102 ±13.038
3	103.6±8.905	105.8 ±11.432	105.4±15.01	104.6 ±3.794	104.6±12.837
5	107.4 ±8.988	110.2 ±11.755	108.4 ±15.757	106.8 ±14.618	106.8 ±13.084
7	111.6	113.6 ±10.430	110 ±15.313	110 ±15.132	109 ±13.490

	±8.961				
9	115.2 ±8.438	117.2 ±9.984	112 ±14.832	114.2 ±15.927	110.8 ±13.535
11	119 ±7.014	120.6 ±9.423	115 ±14.370	116 ±15.779	115 ±14.577
13	123.2 ±5.674	123.2 ±9.418	116.8 ±14.532	120 ±11.726	121 ±15.165
15	127 ±4.847	125.4 ±9.208	119.4 ±14.536	123 ±12.51	124.4 ±15.126
17	130.8 ±3.768	127.6 ±8.443	120.8 ±14.582	125.6 ±12.817	127.4 ±15.868
19	134.6 ±3.715	131.6 ±8.619	123.8 ±14.720	129.2 ±13.367	131.2 ±16.068
21	139 ±3.807	136.4 ±9.838	126.8 ±14.532	133.6 ±14.570	134.8 ±17.181
23	142.4 ± 3.507	139.2 ± 9.959	133.2±12.716	136.8 ±14.254	139.6 ±16.056
26	144.6 ± 3.847	143.6 ± 10.597	138.4±11.717	136.6 ±14.4672	144 ±16.309
28	147.4 ± 3.781	145.2 ± 9.884	140.2 ±11.819	144.2 ±14.906	146.4 ±16.456

The p value was not <0.05 in terms of body weights. So here is no significant difference in the body weights of the rats of control group and experimental group.

Table: 3.26 Hematological examination of rats of control group

Where, the control group was treated with normal saline water.

Parameters	**Control 1**	**Control 2**	**Control 3**	**Control 4**	**Control 5**	**Avrg**	**S.D.**
Hemoglobin(gm/dl)	12.5	12.2	12.7	12.9	12	12.46	0.364
ESR(in 1st hour mm)	3	2	2	4	5	3.2	1.303
Total count							
White blood cell/cumm	4600	5700	4600	6200	4400	5100	800
Red blood cell(million/cumm)	6.21	6.5	6.4	6.68	6.38	6.434	0.172
Platelate count/cumm	6,77,000	588000	694000	561000	582000	606250	59634.3
Differential leucocyte count							
Neutrophil	14%	16%	18%	14%	17%	16%	0.017
Lymphocytes	82%	80%	80%	83%	80%	81%	0.014
Monocyte	3%	2%	1%	2%	2%	2%	0.007
Eosinophil	1%	2%	1%	1%	1%	1%	0.004
Basophil	0.40%	0.20%	0%	0.50%	30%	6.22%	0.132
Hct. /PCV	38.50%	37.80%	39%	39.90%	37%	38.38%	0.012
MCV (fL)	57.2	56.9	58.8	59.7	57.5	5802%	1.186
MCH (pg)	18.6	18.4	18.9	19.3	18.8	18.8	0.339
MCHC (g/dL)	31.6	32.5	33.1	32.8	32.7	32.54	0.568
RDW-SD (fL)	32.4	32.9	33.4	33.7	37.50	33.98	2.029
RDW-CV	17.5	18.7	17.2	16.9	19.8	18.02	1.207
PDW (fL)	13.8	14.6	14.8	14.3	14.2	14.34	0.384
MPV (fL)	8.2	8	7.9	7.3	7.1	7.7	0.474
PCT	0.42%	0.44%	0.43%	0.40%	41%	8.54%	0.181

Table: 3.27 Hematological examination of rats of leaf sample at 250mg/kg dose.

Parameters	**Rat 1**	**Rat 2**	**Rat 3**	**Rat 4**	**Rat 5**	**Average**	**S.D.**
Hemoglobin(gm/dL)	12.8	13.2	12.5	12.8	13	12.86	0.260
ESR(mm. in 1st hour)	2	4	2	3	2.5	2.7	0.836
Total count							
White blood cell/cumm	4200	3500	2600	3300	4100	3540	650.384
Red blood cell(million/cumm)	5.86	6.4	6.9	6.2	5.5	6.172	0.531
Platelate count/ cumm	415000	567000	624000	523000	418000	509400	92061.39
Differential leucocyte count							
Neutrophil	7%	5%	7%	5%	3%	5%	0.016
Lymphocytes	91%	91%	90%	92%	95%	92%	0.019
Monocyte	1%	1%	1%	1%	1%	1%	0
Eosinophil	1%	1%	1%	1%	1%	1%	0
Basophil	0.83%	2%	1.50%	1%	0.94%	1.31%	0.004
Hct. /PCV	40.10%	42.50%	38.20%	39.50%	41.80%	40.42%	0.017
MCV (fL)	58.8	62.7	57.8	60.5	63.8	60.72	2.533
MCH (pg)	19.2	19.2	18.8	18.5	18.8	18.9	0.3
MCHC (g/dL)	32.8	31.5	31.7	30.8	31.4	31.64	0.730
RDW-SD (fL)	32.6	34	38.4	36.8	32.8	34.92	2.567
RDW-CV	18.40%	16.90%	18.30%	17.40%	16.50%	17.50%	0.008
PDW (fL)	13.1	11.1	12	11.6	11.2	11.8	0.809
MPV (fL)	8.1	7.3	7.4	7.3	7.4	7.5	0.339
PCT	0.35%	0.45%	0.40%	0.38%	0.36%	0.39%	0.000

Table: 3.28 Hematological examination of rats of leaf sample at 500mg/kg dose.

Parameters	Rat 1	Rat 2	Rat 3	Rat 4	Rat 5	Avrg	S.D.
Hemoglobin(gm/dL)	13	12.2	12.6	12.4	12.7	12.58	0.303
ESR(mm. in 1st hour)	4	8	3	4	6	5	2
Total count							
White blood cell/per cumm	2400	3000	5100	2100	1900	2900	1298.075
Red blood cell(million/cumm)	4.85	6.6	4.28	6.24	4.24	5.242	1.109
Platelate count/cumm	615000	652000	764000	655000	792000	695600	77487.42
Differential leucocyte count							
Neutrophil	6%	4%	5%	10%	6%	6%	0.022
Lymphocytes	91%	93%	94%	87%	92%	91%	0.027
Monocyte	1%	1%	1%	1%	1%	1%	0
Eosinophil	1%	1%	1%	1%	1%	1%	0
Basophil	0.96%	0.46%	0.27%	1%	0.29%	0.60%	0.003
Hct. /PCV	42.30%	35.50%	38.20%	36.00%	38.10%	38.02%	0.026
MCV (fL)	61.5	53.8	54.2	52.3	58.2	56	3.770
MCH (pg)	19.9	18.5	18.8	18%	19.2	15.3152	8.479
MCHC (g/dL)	33.4	34.4	33.9	32%	33.6	27.1248	14.986
RDW-SD (fL)	31.9	30	31.2	31%	33.5	25.3814	14.074
RDW-CV	16.60%	16.40%	16.80%	17.30%	18.30%	17.08%	0.007
PDW (fL)	15	16	15.7	15	14.6	15.26	0.572
MPV (fL)	7.9	7.2	7.9	7.2	7.8	7.6	0.367
PCT	0.46%	0.46%	0.54%	0.46%	0.57%	0.50%	0.000

Table: 3.29 Hematological examination of the rats of stem sample at 250mg/kg dose.

Parameters	Rat 1	Rat 2	Rat 3	Rat 4	Rat 5	Avrg	SD
Hemoglobin gm/dL	11.9	11.4	13.5	14.5	12.1	12.68	1.146
ESR(mm. in 1st hour)	3	6	2	2	4	3.4	1.496
Total count							
White blood cell/ cumm	5900	4800	3100	2800	3600	4040	1153.43
Red blood cell(million/cumm)	5.8	3.8	6.2	7.5	6	5.86	1.189
Platelate count/ cumm	384000	263000	573000	612000	681000	502600	155064.6
Differential leucocyte count							
Neutrophil	8%	7%	6%	7%	4%	6%	0.013
Lymphocytes	88%	90%	92%	90%	94%	91%	0.020
Monocyte	1%	1%	1%	1%	1%	1%	0
Eosinophil	1%	1%	1%	1%	1%	1%	0
Basophil	2%	1%	2.30%	1.21%	0.39%	1%	0.006
Hct. /PCV	35.10%	33%	41.20%	44.60%	36%	37.98%	0.042
MCV (fL)	51.80	61.30	58.50	58.90	53.50	56.80	3.562
MCH (pg)	18.20	24.10	19.2	19.3	18.20	19.80	2.200
MCHC (g/dL)	33.8	34.00	33.4	32.8	34	33.60	0.456
RDW-SD (fL)	30.5	31.20	32.2	35.5	32.1	32.30	1.716
RDW-CV	16%	17.10%	17.60%	18.20%	17.20%	17%	0.007
PDW (fL)	15.6	15.40	16.9	13.4	16.8	15.62	1.265
MPV (fL)	7.2	9.30	8.4	6.9	7%	6.37	3.266
PCT	0.34%	0.24%	0.47%	0.46%	0.51%	0.40%	0.000

Table: 3.30 Hematological examination of the rats of stem sample at 500mg/kg dose.

Parameters	Rat 1	Rat 2	Rat 3	Rat 4	Rat 5	Average	SD
Hemoglobin(gm/dL)	12.7	12.3	10.6	11.8	12	11.88	0.791
ESR(mm. in 1st hour)	3	3.5	2.8	3.4	3	3.14	1.340
Total count							
White blood cell/cumm	3900	2600	2400	3300	3100	3060	594.138
Red blood cell(million/cumm	6.2	6.1	5.45	4.86	6.34	5.79	0.621
Platelate count/cumm	517000	554000	196000	418000	463000	429600	140475.3
Differential leucocyte count							
Neutrophil	10%	14%	7%	5%	9%	9%	0.033
Lymphocytes	87%	82%	87%	88%	85%	86%	0.023
Monocyte	1%	1%	1%	1%	2%	1%	0.004
Eosinophil	1%	1%	1%	1%	1%	1%	0
Basophil	1.30%	2%	4%	5.30%	4.20%	3.36%	0.016
Hct. /PCV	39.40%	37.30%	33%	35.90%	38.40%	36.80%	0.024
MCV (fL)	58.40	59.90	60.60	58.20	59.80	59.38	1.035
MCH (pg)	19	19.70	19.40	18.8	19	19.18	0.363
MCHC (g/dL)	32.2	33	32.10	33.1	33.1	32.70	0.504
RDW-SD (fL)	39.6	35.8	35.60	31	34	35.20	3.120
RDW-CV	18%	18.20%	18%	17.50%	17.50%	18%	0.003
PDW (fL)	12.2	12.3	11.90	13	16.9	13.26	2.074
MPV (fL)	7	6.9	6.40	7.2	7.5	7.00	0.406
PCT	0.39%	0.52%	0.19%	0.38%	0.39%	0.37%	0.001

Table: 3.31 Bio-chemical examination of rats of control group

Parameters	**Control 1**	**Control 2**	**Control 3**	**Control 4**	**Control 5**	**Average**	**S.D.**
RBS(Random blood sugar(mmol/L)	3.1	2.9	4.0	3.8	3.2	3.4	0.474
S. Urea(mg/dL)	20	22	18	20	23	20.6	1.949
S. Creatinine(mg/dL)	0.94	1.14	1.21	0.89	1.05	1.046	0.133
Liver function test							
S. Bilirubin(mg/dl	0.28	0.23	0.2	0.17	0.2	0.216	0.041
SGPT/ALT(U/L)	64	50	74	67	59	62.8	8.983
SGOT/AST(U/L)	102	94	110	94	91	98.2	7.758
Alkaline Phosphate(U/L)	310	284	349	246	294	296.6	37.587
Lipid profile							
S. Cholesterol(g/dL)	80	70	75	72	77	74.8	3.962
S. Triglyceride(g/dL)	47	37	44	44	38	42	4.301
S. HDL-Cholesterol(g/dL)	54	56	50	46	42	49.6	5.727
S.LDL-Cholesterol(g/dL)	17	7	16	18	27	17	7.106
Electrolyte							
Sodium(Na+) (mmol/L)	143	140	148	140	146	143.4	3.577
Potassium(K+) (mmol/L)	5.3	5.4	4.8	4.5	4.3	4.86	0.482
Chloride(Cl-) (mmol/L)	98	101	97	103	98	99.4	2.509

S. Calcium(g/dL)	8.1	7.5	8.6	8	8.5	8.14	0.439
S. Uric acid(g/dL)	2.1	1.8	1.5	2	1.8	1.84	0.230
S. Protein (g/dL)	6.2	6.6	6.3	6.4	6.1	6.32	0.192
S. Albumin(g/dL)	2.9	3.1	3.5	3	2.9	3.08	0.248
S. Globulin (g/dL)	3.3	3.5	2.8	3.4	3.2	3.24	0.275

Table: 3.32 Bio-chemical examination of rats of leaf sample at 250mg/kg

Prameters	**Rat 1**	**Rat 2**	**Rat 3**	**Rat 4**	**Rat 5**	**Avrg**	**S.D.**
RBS(Random blood sugar)	2.9	3.5	3.1	3.3	3.3	3.22	0.228
S. Urea(mg/dL)	18	23	18	20	17	19.2	2.387
S. Creatinine(mg/dL)	0.68	0.82	0.76	0.8	0.71	0.754	0.058
Liver function test							
S. Bilirubin(mg/dl)	0.15	0.18	0.16	0.15	0.16	0.16	0.012247
SGPT/ALT(U/L)	77	90	82	76	63	77.6	9.864076
SGOT/AST(U/L)	124	140	132	137	107	128	13.20984
Alkaline Phosphate(U/L)	255	305	281	290	184	263	47.75458
Lipid profile							
S. Cholesterol(g/dL)	76	82	78	71	67	74.8	5.890
S. Triglyceride(g/dL)	40	38	50	44	35	41.4	5.813
S. HDL-Cholesterol(g/dL)	57	62	47	59	41	53.2	8.843
S.LDL-Cholesterol(g/dL)	11	12	21	20	19	16.6	4.722
Electrolyte							
Sodium(Na+) (mmol/L)	141	145	143	145	148	144.4	2.607
Potassium(K+) (mmol/L)	4	4.4	4	4.2	4.7	4.26	0.296
Chloride(Cl-)	107	102	95	110	113	105.4	7.092

(mmol/L)							
S. Calcium(g/dL)	7.4	8.6	7.3	7.2	7.6	7.62	0.567
S. Uric acid(g/dL)	1.5	2.3	1.9	2.1	1.9	1.94	0.296
S. Protein(g/dL)	6.3	6	6.4	6.3	6.4	6.28	0.164
S. Albumin(g/dL)	3	3.4	3.2	3.3	3.2	3.22	0.148
S. Globulin (g/dL)	3.3	2.6	3.2	2.9	3.2	3.04	0.288

Table: 3.33 Bio-chemical examination of rats of leaf sample at 500mg/kg

Prameters	**Rat 1**	**Rat 2**	**Rat 3**	**Rat 4**	**Rat 5**	**Avrg**	**S.D.**
RBS(Random blood sugar)	3.7	3.1	2	2.8	2.3	2.78	0.668
S. Urea(mg/dL)	24	20	23	18	25	22	2.915
S. Creatinine(mg/dL)	1.26	1.18	1	0.84	1.21	1.098	0.174
Liver function test							
S. Bilirubin (mg/dL)	0.17	0.23	0.15	0.18	0.16	0.178	0.031
SGPT/ALT(U/L)	85	76	62	68	84	75	10
SGOT/AST(U/L)	151	121	96	109	134	122.2	21.393
Alkaline Phosphate(U/L)	386	269	240	194	271	272	70.911
Lipid profile							
S. Cholesterol(g/dL)	78	73	64	70	64	69.8	6.016
S. Triglyceride(g/dL)	37	48	36	43	40	40.8	4.868
S. HDL-Cholesterol(g/dL)	53	55	44	34	52	47.6	8.677
S.LDL-Cholesterol(g/dL)	17	9	13	23	7	13.8	6.418
Electrolyte							
Sodium(Na+) (mmol/L)	150	140	146	152	145	146.6	4.669

Potassium(K+) (mmol/L)	4.6	5.3	4.7	4.3	4.9	4.76	0.371484
Chloride(Cl-) (mmol/L)	109	114	105	95	108	106.2	7.049
S. Calcium(g/dL)	7.8	7.2	8.3	8	8.4	7.94	0.477
S. Uric acid(g/dL)	2.3	1.6	1.9	1.5	1.8	1.82	0.311
S. Protein (g/dL)	6.4	6.8	6.4	6.6	6	6.44	0.296
S. Albumin(g/dL)	3.5	2.8	3.2	3	2.7	3.04	0.320
S. Globulin (g/dL)	2.9	5	3.2	3.6	3.3	3.6	0.821

Table: 3.34 Bio-chemical examination of rats of stem sample at 250mg/kg

Prameters	**Rat 1**	**Rat 2**	**Rat 3**	**Rat 4**	**Rat 5**	**Avrg**	**SD**
RBS(Random blood sugar)	2.7	3.6	3.1	2	3.4	2.96	1.335
S. Urea(mg/dL)	21	17	16	19	22	19	8.084
S. Creatinine(mg/dL)	0.74	0.62	0.79	0.93	1.02	0.82	0.334
Liver function test							
S. Bilirubin (mg/dL)	0.2	0.18	0.16	0.22	0.18	0.188	0.079
SGPT/ALT(U/L)	47	54	35	44	50	46	7.176
SGOT/AST(U/L)	73	82	71	79	91	79.2	7.949
Alkaline Phosphate(U/L)	186	190	176	186	176	184.5	6.418
Lipid profile							
S. Cholesterol(g/dL)	66	70	81	78	85	76	7.842
S. Triglyceride(g/dL)	39	45	39	35	48	41.2	5.215
S. HDL-Cholesterol(g/dL)	47	52	55	58	54	53.2	4.086
S.LDL-Cholesterol(g/dL)	11	9	18	13	21	14.4	4.979
Electrolyte							
Sodium(Na+)	146	151	143	149	146	147	3.082

(mmol/L)							
Potassium(K+) (mmol/L)	4.2	4	4.7	5.2	5	4.62	0.511
Chloride(Cl-) (mmol/L)	102	96	108	104	97	101.4	4.97
S. Calcium(g/dL)	9.1	8.7	8.3	8.8	8.5	8.68	0.303
S. Uric acid(g/dL)	1.4	1.8	1.5	1.9	1.5	1.62	0.216
S. Protein (g/dL)	6.6	6.3	6	6.7	6.4	6.4	0.273
S. Albumin(g/dL)	3	3.2	2.8	2.9	3.1	3	0.158
S. Globulin (g/dL)	3.6	3.1	3.2	3.8	3.3	3.4	0.291

Table: 3.35 Bio-chemical examination of rats of stem sample at 500mg/kg

Prameters	Rat 1	**Rat 2**	**Rat 3**	**Rat 4**	**Rat 5**	**Avrg**	**SD**
RBS(Random blood sugar)	3.8	1.7	2.8	3	3.3	2.92	0.779
S. Urea(mg/dL)	24	20	24	21	20	22.25	2.049
S. Creatinine(mg/dL)	1.1	1.23	1.29	1.15	1.14	1.182	0.076
Liver function test							
S. Bilirubin(mg/dL)	0.19	0.14	0.18	0.16	0.16	0.166	0.019
SGPT/ALT(U/L)	35	40	25	39	41	36	6.557
SGOT/AST(U/L)	72	84	57	73	77	72.6	9.914
Alkaline Phosphate(U/L)	224	185	140	167	164	176	31.249
Lipid profile							
S. Cholesterol(g/dL)	72	62	57	65	63	63.8	5.449
S. Triglyceride(g/dL)	41	37	35	39	38	38	2.236
S. HDL-Cholesterol(g/dL)	51	46	40	44	46	45.4	3.974
S.LDL-Cholesterol(g/dL)	13	9	10	13	9	10.8	2.049
Electrolyte							

Sodium(Na+) (mmol/L)	139	147	151	144	141	144.4	4.774
Potassium(K+) (mmol/L)	4.4	4.8	5	4.7	4.7	4.72	0.216
Chloride(Cl-) (mmol/L)	103	99	100	106	108	103.2	3.858
S. Calcium(g/dL)	8.1	7.9	8.2	8.5	8	8.14	0.230
S. Uric acid(g/dL)	1.6	1.9	2.3	2	1.9	1.94	0.250
S. Protein (Total)(g/dL)	6.2	6.5	6	6.3	6.4	6.28	0.192
S. Albumin(g/dL)	3.4	3.6	3.4	3	3.2	3.32	0.228
S. Globulin (g/dL)	2.8	2.9	2.6	3.3	3.2	2.96	0.288

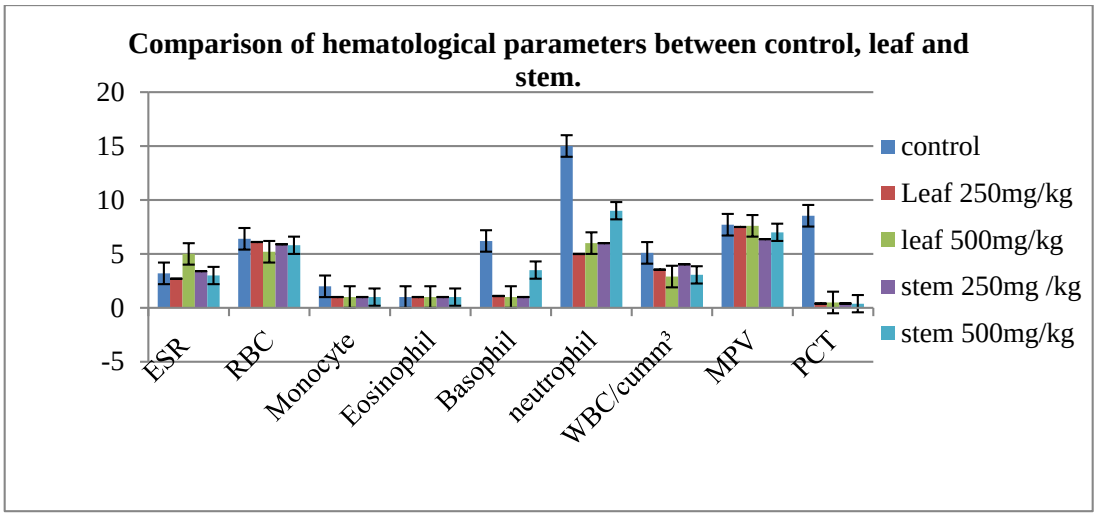
Table: 3.36 Hematological examination of rats after oral administration of control and methanolic leaf and stem extract of *Actephila excelsa* .

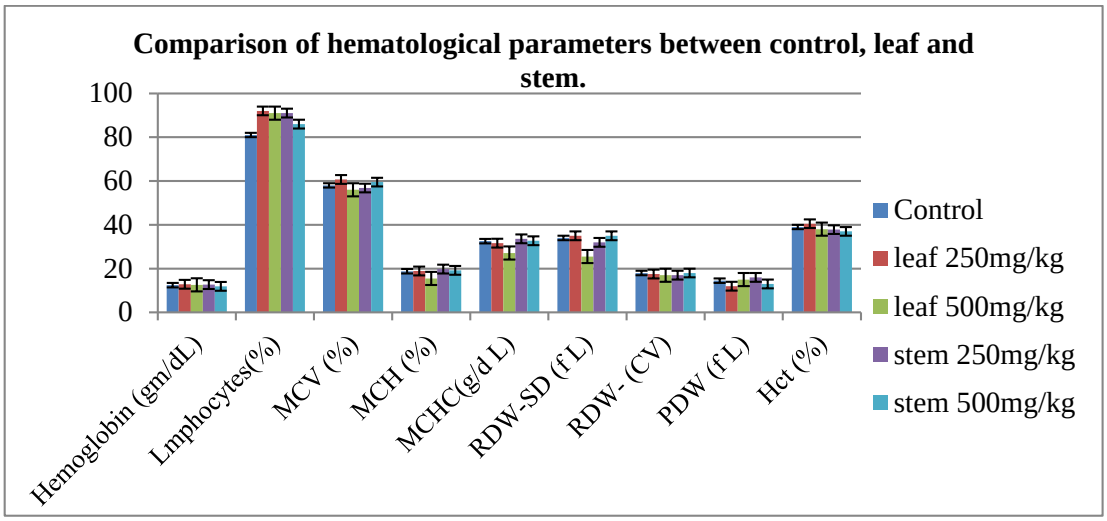
Parameters	**control**	**Leaf 250mg/kg**	**Leaf 500mg/kg**	**Stem 250mg/kg**	**Stem 500mg/kg**
Hemoglobin(gm/dL)	12.46±0.364	12.86±1.146	12.58±0.303	12.68±1.146	11.88±0.791
ESR(mm. in 1st hour)	3.2±1.303	2.7± 1.496	5± 2	3.4± 1.496	3±1.310
Total count					
White blood cell/ cumm	5100± 800	3540±1153.43 *	2900± 1298.075 *	4040± 1153.43 *	3060± 594.138 *
Red blood cell(million/cumm)	6.434± 0.172	6.172± 1.189	5.242± 1.109 *	5.86± 1.189 *	5.79± 0.621*
Platelet count/ cumm	606250± 59634.3	509400± 155064.6	695600± 77487.42 *	502600± 155064.6 *	429600± 140475.3*
Differential leucocyte count					
Neutrophil(%)	16 ± 0.017	5 ± 0.020 *	6±0.022 *	6 ±0.013 *	9±0.033 *
Lymphocytes (%)	81 ± 0.014	92± 0.013 *	91±0.027 *	91±0.020 *	86±0.023 *
Monocyte(%)	2± 0.007	1 ± 0 *	1 ± 0 *	1 ± 0*	1±0.004*
Eosinophil(%)	1 ±0.004	1 ± 0	1 ± 0	1± 0	1±0

Basophil(%)	6.22±0.132	1.31±0.006	0.60± 0.003	1 ± 0.006	3.36±0.016
Hct. /PCV (%)	38.38±0.012	40.42±0.042	38.02±0.026	37.98±0.042	36.80±0.024
MCV (fL) (%)	58.02±1.186	60.72±3.562	56±3.770	56.80±3.562	59.38±1.035
MCH (pg)	18.8 ±0.339	18.9±2.200	15.3152±8.479	19.80±2.200	19.18±0.363
MCHC (g/dL)	32.54±0.568	31.64±0.456	27.1248±14.986	33.60±0.456	32.70±0.504
RDW-SD (fL)	33.98±2.029	34.92±1.7169	25.3814±14.074	32.30±1.716	35.20±3.120
RDW-CV	18.02±1.207	17.50±0.007	17.08±0.007	17±0.007	18±0.003
PDW (fL)	14.34±0.384	11.8±1.265 *	15.26±0.572 *	15.62±1.265 *	13.26±2.074 *
MPV (fL)	7.7± 0.474	7.5± 3.266	7.6± 0.367	6.37± 3.266	7.00± 0.406
PCT(%)	8.54±0.181	0.39±0.000	0.50± 0.000	0.40±0.000	0.37±0.001

Here, the hematological parameters of the rats were calculated as average ± S.D and (*) mark is put on those values having p value (<0.05) which represents significant difference of those particular parameters between control and experimented groups.

Figure: 3.13 Comparison of hematological parameters between control, leaf and stem.





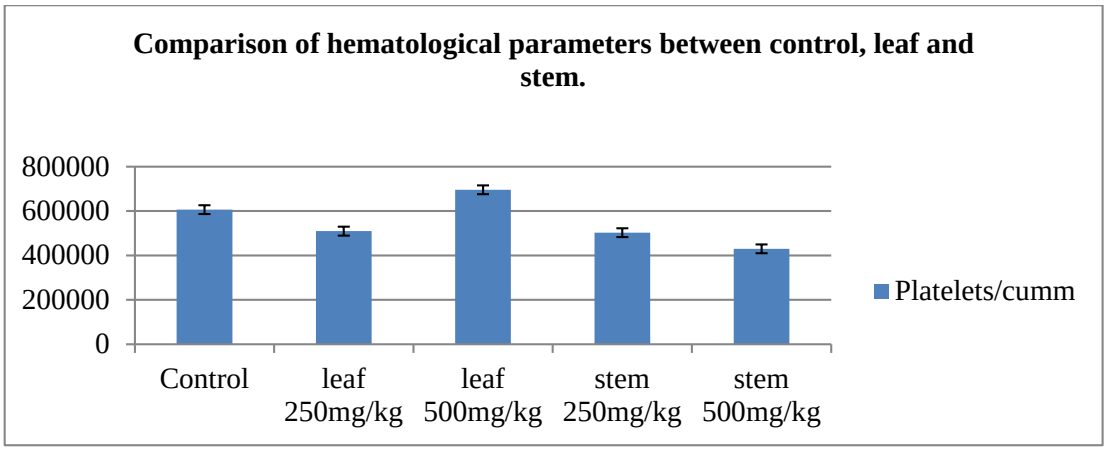
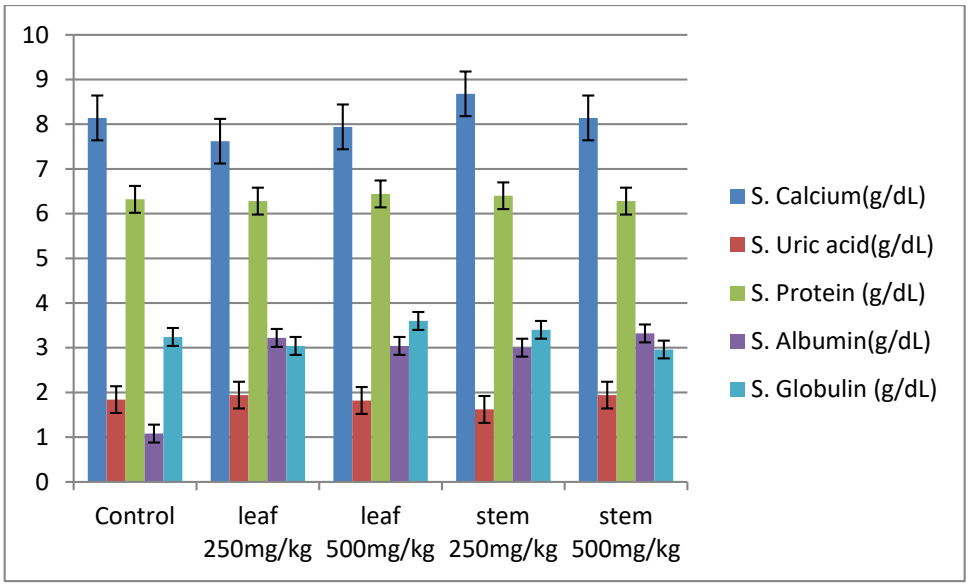
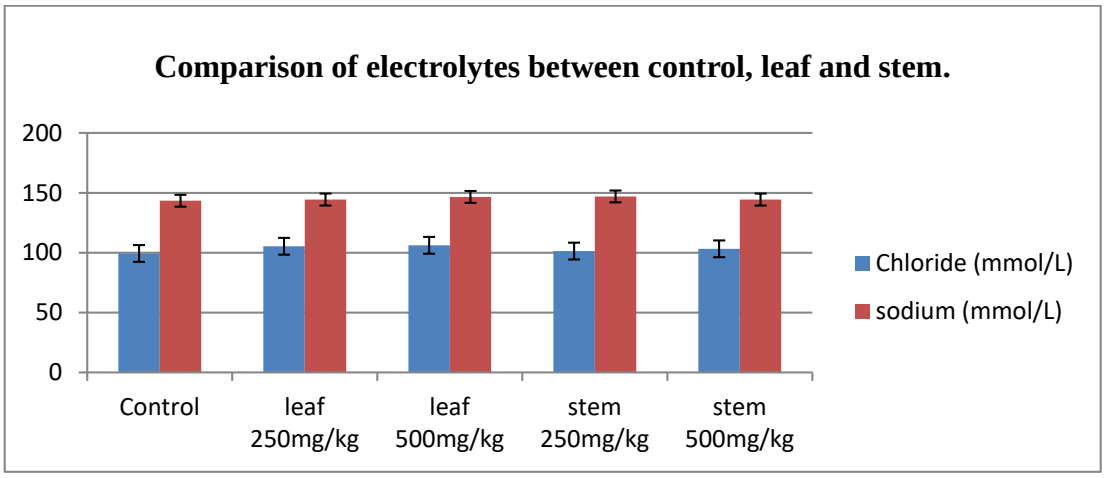


Table: 3.37 Bio-chemical examination of rats after oral administration of control and methanolic leaf and stem extract of *Actephila excelsa* .

Prameters	**Control**	**Leaf sample 250mg/kg**	**Leaf sample 500mg/kg**	**Stem sample 250mg/kg**	**Stem sample 500mg/kg**
RBS(Random blood sugar)	3.4±0.474	3.22±0.228	2.78±0.668	2.96±1.335	2.92±0.779
S. Urea(mg/dL)	20.6±1.949	19.2±2.387	22±2.915	19±8.084	22.25± 2.049
S. Creatinine(mg/dL)	1.046± 0.133	0.754± 0.058 *	1.098± 0.174	0.82± 0.363 *	1.182± 0.076
Liver function test					
S. Bilirubinmg/dL)	0.216±0.041	0.187±0.012	0.178±0.031	0.188±0.079	0.196± 0.019
SGPT/ALT(U/L)	62.8±8.983	77.6±9.864 *	75±10 *	46 ±7.176 *	36± 6.557 *
SGOT/AST(U/L)	98.2±7.758	128±13.209 *	122.2±21.392*	79.2±7.949*	72.6±9.914 *
Alkaline Phosphate(U/L)	296.6± 37.58	263± 47.75458	272± 70.91192	184.5± 6.418723 *	176± 31.249 *
Lipid profile					
S. Cholesterol(g/dL)	74.8±3.962	74.8±5.890	69.8±6.016	76±7.842	63.8±5.449 *
S. Triglyceride(g/dL)	42±4.301	41.4±5.813	40.8±4.868	41.2±5.215	38± 2.236 *
S. HDL-Cholesterol(g/dL)	49.6 5.727	53.2± 8.843	47.6± 8.677	53.2± 4.086	45.4± 3.974
S.LDL-Cholesterol(g/dL)	17 7.106	16.6± 4.722	13.8± 6.418	14.4± 4.979	10.8± 2.049 *
Electrolyte					
Sodium(Na+) (mmol/L)	143.4 3.577	144.4± 2.607	146.6± 4.669	147± 3.082	144.4± 4.774
Potassium (mmol/L)	4.86±0.482	4.26±0.296	4.76±0.371	4.62±0.511	4.72±0.216
Chloride (mmol/L)	99.4±2.509	105.4±7.092	106.2±7.049	101.4±4.979	103.2±3.834
S. Calcium(g/dL)	8.14±0.439	7.62±0.567	7.94±0.477	8.68±0.303	8.14±0.230
S. Uric acid(g/dL)	1.84±0.230	1.94±0.296	1.82±0.311	1.62±0.216	1.94±0.250
S. Protein g/dL)	6.32±0.192	6.28±0.164	6.44±0.296	6.4±0.273	6.28±0.192
S. Albumin(g/dL)	1.08±0.248	3.22± 0.148	3.04±0.320	3±0.158	3.32±0.228
S. Globulin (g/dL)	3.24±0.270	3.04 ± 0.288	3.6±0.821	3.4±0.291	2.96±0.288

Figure: 3.14 Comparison of bio-chemical parameters between control, leaf and stem.





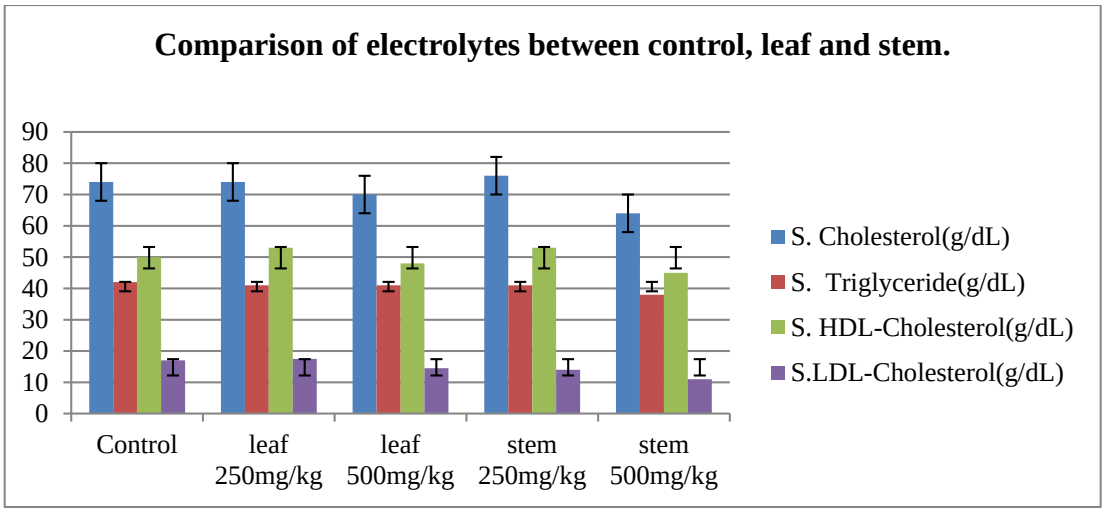
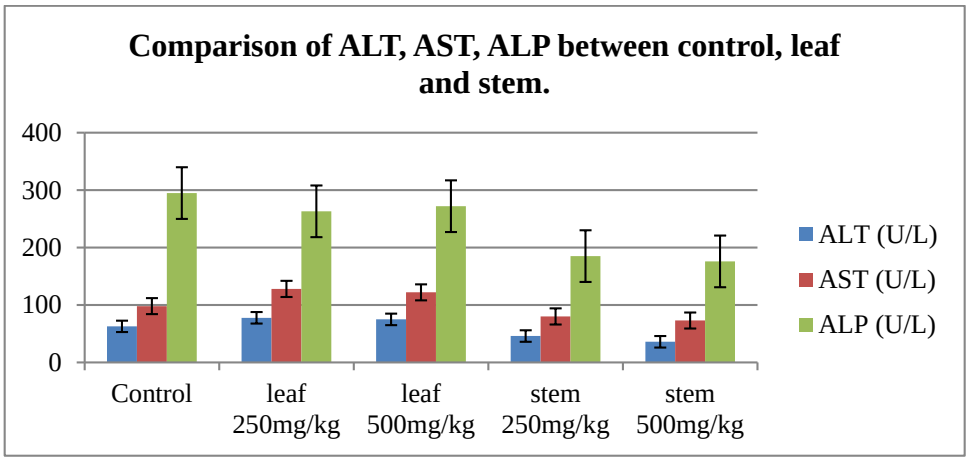
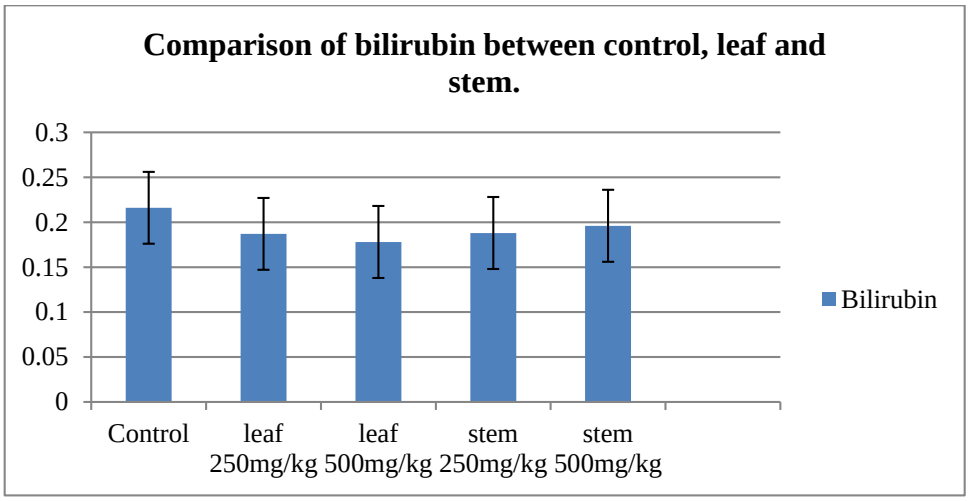
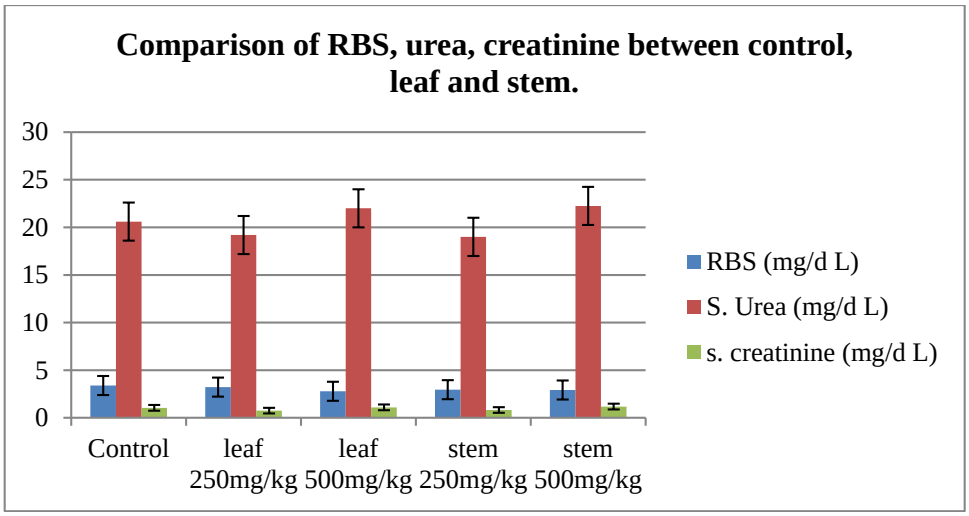


Figure: 3.15 Comparison of bio-chemical parameters between control, leaf and stem.







Chapter 4

Discussion

4. Discussion:

This study was mainly intended to evaluate the antioxidant activity, phytochemical screening, antidiarrheal activity and toxicity of the methanolic extract of the stem and leaf of *Actephila excelsa.* Two third of the plant species contain medicinal values and most of them exerts potential antioxidant activity. Ascorbic acid and also flavonoids are the best exogenous antioxidants which are found in plants(Kasote et al., 2015). Both the stem and leaf extract possess high antioxidant potentials. In DPPH free radical scavenging analysis, the IC_{50} value of stem (115.24) and leaf (92.929) was much higher than the standard ascorbic acid (75.688).Moreover; the percentage of inhibition was also higher in terms of leaf extract than stem which crossed the value of standard with the decrease of concentration. The total phenolic content of the stem and leaf was found 94.8 and 382.75(mg of GAE/gm of extract) respectively, which were also satisfactory values. The total flavonoid content of the methanolic extract of stem and leaf was increased with the increase of concentration. There was gradual increase of flavonoid content from 118.83 to 247.83 (mg of Quercetin/gm of extract) for stem and 104.11 to 231.38 (mg of Quercetin/gm of extract) for leaf with the rising of concentration from 200 µg/mL to 600 µg/mL. So, it can be clearly understood that for the entire antioxidant analysis performed for the project, the leaf and stem extract showed quite acceptable antioxidant capacity. Therefore, this medicinal plant can be used for preparing antioxidants supplement as it is enriched of potential antioxidant capacity.

After performing the phytochemical screening, the methanolic extract of leaf and stem of the plant confirmed the presence of carbohydrate, tannins, saponins, flavonoids and alkaloids. No trace amount of protein or steroids were present in the leaf and stem extract. The presence of alkaloids, tannins and flavonoids in plant extracts reveal that it can be used as potential immunostimulants. Presences of phytochemicals in the plants protect us from different types of diseases. Flavonoid content can reduce the instances of myocardial infarction and thus the mortality rate up to 25% (Lakshmi, Pandey, Puri, Saxena, & Saxena, 2003).

The plant leaf also conferred significant antidiarrheal activity. The % of inhibition of diarrhea using castor oil induced method was 33.33 and 53.65 respectively for 200mg/kg and 400mg/kg extract of leaf. Moreover, the % of inhibition of diarrhea using

$MgSO_4$induced diarrhea was 9.55 and 33.82 respectively for 200mg/kg and 400mg/kg extract of leaf. Thus, % of inhibition of diarrhea using the leaf extract at 400mg/kg dose is slightly higher than the standard which is 52.03 for castor oil induced method and 38.24 for $MgSO_4$ induced method. Loperamide HCl was considered as standard that is traditionally used to treat both acute and chronic diarrhea associated with inflammatory bowel disease. As the extracts show higher percentage of inhibition, it is valid to use the plant leaf extract as potential antidiarrheal medicine.

According to the organization for economic cooperation and development (OECD) panel of experts' acute toxicity is the undesirable effect taking place within a short time of (oral) administration of a particular dose of a substance or multiple doses given within a span of 24 hours. The reason of studying acute toxicity is to determine the LD_{50} values which helps in indicating the safe doses range at which there is no destructive or lethal effect on the treated animal. In acute toxicity, the rats were treated with both the leaf and stem extracts for 14 days at 1000mg/kg dose. There was no sign of mortality or toxicity after the administration of the extracts for the period of 14 days. Also no significant changes in body weight were observed. So, it gives the indication that the median lethal doses (LD_{50}) of the leaf and stem extracts would not be less than or equal to 1000mg/kg.

From the table 3.19 the rats which were treated with 250mg/kg stem and 500mg/kg of both leaf and stem extracts showed significantly lower weights in terms of lungs, heart, liver, pancreas, spleen, kidneys than the organ weights of the control. The rats which were treated with 250mg/kg leaf extract also showed the same results except heart and spleen. So, almost all the organs decrease their weight significantly after being treated with the plant extracts

After the administration of the leaf and stem extracts, there was no significant changes in the body weight of control and experimental rats. The p value was greater than 0.05 for the treated rats. There was gradual insignificant ($p>0.05$) increase in the body weights with days. So at the last day, (28^{th} day) the body weights of all the rats were little bit higher than the first day.

Hematological parameters index are of great importance because of its ability to evaluate the toxic potentials(Sunmonu & Oloyede, 2010). This is also useful to explain the effects of the plant extract on different blood cells.Reports have suggested that this hematological investigation is more precise, reliable and sensitive for diagnosis of diseases(Ighodaro,

Innih, Vincent Ogedengbe, & Amamina, 2016).From table 3.34, it can be seen that the stem extract of 250mg/kgbw and 500mg/kgbw showed significance decrease in WBC, RBC, Platelet, neutrophil, monocyte, and significant increase in lymphocytes count than the count of rats of control group. PDW was significantly increased by 250mg/kgbw and decreased by 500mg/kgbw. Furthermore, WBC, neutrophil, monocyte, were decreased significantly by the both doses of leaf extracts. Whereas, leucocytes count was significantly increased than control. Platelets count and PDW count was significantly increased and RBC count was decreased by 500mg/kgbw leaf extract. If there is a reduction in the platelets count it indicates that the blood cannot carry proper oxygen. It also gives an indication of thrombopoietin (McLellan, McClelland, & Walsh, 2003).So the decreased platelets count by the administration of stem extract of 250mg/kgbw and 500mg/kgbw may decrease the capacity of carrying oxygen of blood. Decrease in RBC count indicates that there may be instability in the osmoregulation of the blood cells or blood cells may undergo any oxidative injury. This incident may have been caused by the lysis of blood cells(Sule, Elekwa, & Ayalogu, 2013). The observed reduction in the RBC count by the administration of stem extracts indicates that there may be shrinkage or destruction of RBC. Through differential white blood cell counts, the capacity of an organism to eradicate infection can be estimated. When there is a significant increase in the circulating leukocytes it rarely indicates that it happens because of the increased count of all leucocytes (H Jorum & M Piero, 2016). Here, WBC count was decreased significantly which indicates that the plant does not have any effect to treat infection. The result also shows reduction in neutrophil count that gives a clear indication that leucopenia can be caused by the administration of the plant extracts (Baker &Silverton, 1998). There are some effector cells in the body to boost up the immune system. Lymphocytes are considered to the major effector cells. So, increased levels of lymphocytes mainly develop the effector cells of the immune system. The blood cell, platelets contributes in coagulation system.For the proper coagulation of blood, the platelets require to be in appropriate size, number and shape. The increase in the platelet counts indicates the stimulatory effects of it in thrombopoietin (Li, Xia, & Kuter, 1999). So, the significant increase in the platelets count demonstrates that the leaf extract possess phytochemicals which is responsible for thrombopoietin stimulation. So, this leaf extract can be used therapeutically in thrombocytopenia. MPV and PDW are simple platelet index. During the

activation of platelets , both of the parameters are increased(Vagdatli et al., 2010). So, the leaf plant extract (500mg/kg) and stem extract (259mg/kg) can be used as potential anticoagulant. There were not any significant changes in the other hematological parameters. By the administration of methanolic extract of leaf and stem at 250mg/kgbw, creatinine level was significantly decreased than control. Though creatinine was decreased than the control group still it was within the normal range of creatinine level. Urea and Uric acid are the biomarkers of kidney. Increase of these markers in body indicates the renal damage (Grotto & Noronha, 2004). There was no significant difference in urea and uric acid level. This reveals that the leaf and stem extracts has no adverse effects on the kidney. Alkaline Phosphate, SGPT, SGOT were decreased by both of the doses of stem while cholesterol, triglycerides, LDL were decreased significantly by the stem extract at 500mg/kgbw dose. These stem extracts can be used to lower the levels of SGOT and SGPT. Increased amount of these two markers indicates that the liver may be damaged. After the administration of the two doses of leaf extract the level of SGPT and SGOT was increased significantly than control. So the leaf extract at both doses can potentially damage the liver. From the lipid profile test, it was obtained that the stem extract at high dose (500mg/kg) potentially decrease the cholesterol level. The harmful triglycerides and LDL were also decreased at a significant level. So, the stem extract can be used as a cholesterol lowering agent. Apart from these differences, the other parameters were not significantly different from control.

Chapter 5

Conclusion

5. Conclusion:

In the developed countries, the use of medicinal plants or herbal medicines has extended widely in the last half of the twentieth century. That is why the interest of conducting research on medicinal plants is increasing day by day. For this project, the medicinal plant *Actephila excelsa* was preferred to discover some constructive properties such as antioxidant potentials, phytochemical screening, antidiarrheal activity and toxicological activity. After conducting the research, it can be decided that the stem and leaf extract of *Actephila excelsa* confirmed different biological activities such as antioxidant, antidiarrheal as well as presence of some important phytochemicals. This plant extract can be used in reducing the oxidative stress and frequency of diarrhea significantly. Moreover, through the toxicological study, it can be decided that the plant has some contributory effects to treat several disease. The leaf extract can be used in thrombocytopenia as it shows increased platelets count. The stem extracts in high dose is very useful in lowering the cholesterol at moderate level.

Group of different essential evaluations such as analgesic activity, antimicrobial activity, cytotoxic activity is further need to be performed by using this plant. As this is the first report on different biological activities and toxicological study, this study can keep crucial role in modern medicine development.

Chapter 6

References

6. References:

Abd El-Baky, H. H., El Baz, F. K., & El-Baroty, G. S. (2009). Enhancement of antioxidant production in Spirulina platensis under oxidative stress. *Acta Physiologiae Plantarum*, *31*(3), 623–631. https://doi.org/10.1007/s11738-009-0273-8

Ajlan, A. (2016). Natural Products : An Indian Journal Medicinal Plants : A Review. *Natural Products*, *12*(3), 1–6.

Ali, H., Houghton, P. J., & Soumyanath, A. (2006). α-Amylase inhibitory activity of some Malaysian plants used to treat diabetes; with particular reference to Phyllanthus amarus. *Journal of Ethnopharmacology*, *107*(3), 449–455. https://doi.org/10.1016/j.jep.2006.04.004

Bardhan, S., Ashrafi, S., & Saha, T. (2018). iMedPub Journals Commonly Used Medicinal Plants in Bangladesh to treat Different Infections, 1–4.

Brewer, M. S. (2011). Natural Antioxidants : Sources , Compounds , Mechanisms of Action , and Potential Applications R : *Comprehensive Reviews in Food Science and Food Safety*, *10*. https://doi.org/10.1111/j.1541-4337.2011.00156.x

Carocho, M., & Ferreira, I. C. F. R. (2013). A review on antioxidants, prooxidants and related controversy: Natural and synthetic compounds, screening and analysis methodologies and future perspectives. *Food and Chemical Toxicology*, *51*(1), 15–25. https://doi.org/10.1016/j.fct.2012.09.021

Chanda, S., & Dave, R. (2009). In vitro models for antioxidant activity evaluation and some medicinal plants possessing antioxidant properties : An overview. *African Journal of Microbiology Research*, *3*(13), 981–996. https://doi.org/10.1007/s13197-011-0276-5

Davidson-hunt, I. (2000). Ecological Ethnobotany: Stumbling Toward New Practices and Paradigms. *MASA*, *Volume 16*(1), 1–13.

Duffus, J., & Worth, H. G. J. (2013). *Fundamental Toxicology*. *Journal of Chemical Information and Modeling* (Vol. 53). https://doi.org/10.1017/CBO9781107415324.004

Edeoga, H. O., Okwu, D. E., & Mbaebie, B. O. (2005). Phytochemical constituents of some Nigerian medicinal plants. *African Journal of Biotechnology*, *4*(7), 685–688. https://doi.org/10.5897/AJB2005.000-3127

Fang, S. H., Rao, Y. K., & Tzeng, Y. M. (2008). Anti-oxidant and inflammatory mediator's growth inhibitory effects of compounds isolated from Phyllanthus urinaria. *Journal of*

Ethnopharmacology, *116*(2), 333–340. https://doi.org/10.1016/j.jep.2007.11.040

Gill, S. S., & Tuteja, N. (2010). Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. *Plant Physiology and Biochemistry*, *48*(12), 909–930. https://doi.org/10.1016/j.plaphy.2010.08.016

Greenwood, B., & Wise, J. (2001). Intermittent drugs seen highly protective against malaria. *World Health*, *79*(7).

Grotto, H. Z. W., & Noronha, J. F. A. (2004). Platelet larger cell ratio (P-LCR) in patients with dyslipidemia. *Clinical and Laboratory Haematology*, *26*(5), 347–349. https://doi.org/10.1111/j.1365-2257.2004.00634.x

Gruenwald, J., Brendler, T., & Jaenicke, C. (2000). *Bitter Orange*. *PDR for Herbal Medicines*.

Guerrant, R. L., Gilder, T. Van, Steiner, T. S., Thielman, N. M., Slutsker, L., Tauxe, R. V, … Pickering, L. K. (2001). Practice Guidelines for the Management of Infectious Diarrhea. *Clinical Infectious Diseases*, *32*, 33150. https://doi.org/10.1086/318514

Gutiérrez M, Carmona U, Vallejos G, A. L. (2012). Antifungal activity of tetrahydroquinoline against some Phytopathogenic fungi. *Zeitschrift Für Naturfurschung C, Journal of Biosciences.*, *67*(11–12), 551–556.

H Jorum, O., & M Piero, N. (2016). Haematological Effects of Dichloromethane-Methanolic Leaf Extracts of Carissa edulis (Forssk.) Vahl in Normal Rat Models. *Journal of Hematology & Thromboembolic Diseases*, *04*(01), 1–8. https://doi.org/10.4172/2329-8790.1000232

Hoffmann, P., Kathriarachchi, H., & Wurdack, K. J. (2006). A phylogenetic classification of Phyllanthaceae (Malpighiales; Euphorbiaceae sensu lato). *Kew Bulletin*, *61*(1), 37–53. Retrieved from http://www.jstor.org/stable/10.2307/20443245

Ighodaro, I., Innih, S., Vincent Ogedengbe, S., & Amamina, L. (2016). Chronic Toxicity Studies of Aqueous Leaf Extract of <i>Voacanga africana</i> in Wistar Rats. *Journal of Applied Sciences and Environmental Management*, *19*(4), 639. https://doi.org/10.4314/jasem.v19i4.11

Islam, A., Selvan, T., Mazumder, U., & Gupta, M. (2008). Antitumour effect of phyllanthin and hypophyllanthin from Phyllanthus amarus against Ehrilch ascites carcinoma in mice. *Pharmacologyonline*, *807*, 796–807. Retrieved from

Jacquart, D. (2008). Islamic pharmacology in the middle ages: Theories and substances. *European Review*, *16*(2), 219–227. https://doi.org/10.1017/S1062798708000215

Kasote, D. M., Hegde, M. V., & Katyare, S. S. (2013). Mitochondrial dysfunction in psychiatric and neurological diseases: Cause(s), consequence(s), and implications of antioxidant therapy. *BioFactors*, *39*(4), 392–406. https://doi.org/10.1002/biof.1093

Kasote, D. M., Katyare, S. S., Hegde, M. V., & Bae, H. (2015). Significance of antioxidant potential of plants and its relevance to therapeutic applications. *International Journal of Biological Sciences*, *11*(8), 982–991. https://doi.org/10.7150/ijbs.12096

Kelly, K. (2009). *History of medicines-The Middle Ages 500-1450*.

Krishnaiah, D., Sarbatly, R., & Nithyanandam, R. (2011). A review of the antioxidant potential of medicinal plant species. *Food and Bioproducts Processing*, *89*(3), 217–233. https://doi.org/10.1016/j.fbp.2010.04.008

Krithika, R., Mohankumar, R., Verma, R. J., Shrivastav, P. S., Mohamad, I. L., Gunasekaran, P., & Narasimhan, S. (2009). Isolation, characterization and antioxidative effect of phyllanthin against CCl4-induced toxicity in HepG2 cell line. *Chemico-Biological Interactions*, *181*(3), 351–358. https://doi.org/10.1016/j.cbi.2009.06.014

Kritikar, K. R., & Basu, B. D. (1918). *Important medicinal plants*. : India: Published by Sudhindra Nath Basu, M.B. Panini Office, Bhuwanéswari Asrama, Bahadurganj.

Kurmukov, A. G. (2013). Phytochemistry of medicinal plants. *Medicinal Plants of Central Asia: Uzbekistan and Kyrgyzstan*, *1*(6), 13–14. https://doi.org/10.1007/978-1-4614-3912-7_4

Lakshmi, V., Pandey, K., Puri, A., Saxena, R. P., & Saxena, K. C. (2003). Immunostimulant principles from Curculigo orchioides. *Journal of Ethnopharmacology*, *89*(2–3), 181–184. https://doi.org/10.1016/S0378-8741(03)00160-0

Li, J., Xia, Y., & Kuter, D. J. (1999). Interaction of thrombopoietin with the platelet c-mpl receptor in plasma: Binding, internalization, stability and pharmacokinetics. *British Journal of Haematology*, *106*(2), 345–356. https://doi.org/10.1046/j.1365-2141.1999.01571.x

LONDHE, J. S., DEVASAGAYAM, T. P. A., FOO, L. Y., & GHASKADBI, S. S. (2009). Radioprotective Properties of Polyphenols from Phyllanthus amarus Linn. *Journal of Radiation Research*, *50*(4), 303–309. https://doi.org/10.1269/jrr.08096

Lutterodt, G. D. (1992). Inhibition of microlax-induced experimental diarrhoea with narcotic-like extracts of Psidium guajava leaf in rats. *Journal of Ethnopharmacology*, *37*(2), 151–157. https://doi.org/10.1016/0378-8741(92)90073-Z

Mabeku Laure B., K., Kouam, J., Beng V., P., Ngadjui, B. T., Fomum, Z. T., & Etoa, F. X. (2007). Evaluation of antimicrobial activity of the stem bark of Cylicodiscus gabunensis (mimosaceae). *African Journal of Traditional, Complementary and Alternative Medicines*, *4*(1), 87–93.

Mao, X., Wu, L. F., Guo, H. L., Chen, W. J., Cui, Y. P., Qi, Q., … Zhang, L. Z. (2016). The Genus Phyllanthus: An Ethnopharmacological, Phytochemical, and Pharmacological Review. *Evidence-Based Complementary and Alternative Medicine*. https://doi.org/10.1155/2016/7584952

Mazumder, A., Mahato, A., & Mazumder, R. (2006). Antimicrobial potentiality of Phyllanthus amarus against drug resistant pathogens. *Natural Product Research*, *20*(4), 323–326. https://doi.org/10.1080/14786410600650404

McLellan, S. A., McClelland, D. B. L., & Walsh, T. S. (2003). Anaemia and red blood cell transfusion in the critically ill patient. *Blood Reviews*, *17*(4), 195–208. https://doi.org/10.1016/S0268-960X(03)00018-3

Nelson, D. M., & Cox, M. M. (2005). *Lehninger Principles of Biochemistry* (4th ed.). New York: W.H. Freeman and Company.

Olaniyan, J. M., Muhammad, H. L., Makun, H. A., Busari, M. B., & Abdullah, A. S. (2016). Acute and sub-acute toxicity studies of aqueous and methanol extracts of Nelsonia campestris in rats. *Journal of Acute Disease*, *5*(1), 62–70. https://doi.org/10.1016/j.joad.2015.08.006

Omidiran, M. O., Baiyewu, R. A., Ademola, I. T., Fakorede, O. C., Toyinbo, E. O., Adewumi, O. J., & Adekunle, E. A. (2012). Phytochemical analysis, nutritional composition and antimicrobial activities of white mulberry (Morus alba). *Pakistan Journal of Nutrition*, *11*(5), 456–460. https://doi.org/10.3923/pjn.2012.456.460

Petrovska, B. B. (2012). Historical review of medicinal plants ' usage. *Pharmacognosy Reviews*, *6*(11), 1–6. https://doi.org/10.4103/0973-7847.95849

Pryor, W. A. (2004). *Phytochemicals in health and disease*. *Free Radical Biology and Medicine* (Vol. 37). https://doi.org/10.1016/j.freeradbiomed.2004.08.005

Recio, M. del C., Giner, R. M., Máñez, S., & Rios, J. L. (1995). Structural requirements for the anti-inflammatory activity of natural triterpenoids. *Planta Medica*, *61*, 182–185.

Sadi, A. (2012). প্রাণকাকলি: বাংলাদেশেরঔষধিগাছেরএকটিবিস্তারিতপাঠ, Medicinal Plants of Bangladesh. প্রাণকাকলি. Retrieved from anupsadi.blogspot.com/2012/12/medicinal-plants-of-bangladesh_24.html

Saha, D., & Paul, S. (2012). Study of antidiarrhoeal activity of two Bangladeshi medicinal plants in castor-oil induced diarrhoea. *Research Journal of Pharmacy and Technology*, *5*(6), 800–804. https://doi.org/10.1016/S0378-8741(00)00379-2

Sarin, B., Verma, N., Martín, J. P., & Mohanty, A. (2014). An overview of important ethnomedicinal herbs of phyllanthus species: Present status and future prospects. *The Scientific World Journal*, *2014*. https://doi.org/10.1155/2014/839172

Sasidharan, N. (2004). *Biodiversity documentation for Kerala. Part 6: Flowering plants*. KFRI.

Sehwag, S., & Das, M. (2014). Nutritive, therapeutic and processing aspects of Jamun, Syzygium cuminii (L.)Skeels- an overview. *Indian Journal of Natural Products and Resources*, *5*(4), 295–307.

Sewell, R. D. E., & Rafieian-kopaei, M. (2014). The history and ups and downs of herbal medicines usage. *Journal of HerbMed Pharmacology Journal Homepage*, *3*(1), 1–3.

Shin, M. S., Kang, E. H., & Lee, Y. I. (2005). A flavonoid from medicinal plants blocks hepatitis B virus-e antigen secretion in HBV-infected hepatocytes. *Antiviral Research*, *67*(3), 163–168. https://doi.org/10.1016/j.antiviral.2005.06.005

Siddique, A., Akhtar, N., Khan, M. S., Anwar, M., Jan, S., & Khan, W. M. (2016). Diversity , distribution and indigenous uses of medicinal plants of district karak khyber pakhtunkhawa pakistan. *Pakistan Journal of Weed Science Research*, *22*(2), 317–327.

Sies, H. (1986). Biochemistry of Oxdative Stress. *Angewandte Chemie - International Edition*, *25*, 1058–1071.

Sies, H. (1997). Oxidative stress: Oxidants and antioxidants. *Experimental Physiology*, *82*(2), 291–295. https://doi.org/10.1113/expphysiol.1997.sp004024

Singh, R. (2015). Medicinal plants : A review. *Journal of Plant Sciences*, *3*, 50–55. https://doi.org/10.11648/j.jps.s.2015030101.18

Srivastava, A. K. (2018). *Significance of medicinal plants in human life*. *Synthesis of*

Medicinal Agents from Plants. Elsevier Ltd. https://doi.org/10.1016/B978-0-08-102071-5/00001-5

Sule, O. ., Elekwa, I., & Ayalogu, E. . (2013). Effect of Aqueous Leaf Extract of Acalypha wilkesiana on Hematological Parameters in Male Wistar Albino Rats. *International Journal of Biological & Medical Research*, *3*(1), 465–471.

Sunmonu, T. O., & Oloyede, O. B. (2010). Performance and haematological indices in rats exposed to monocrotophos contamination. *Human and Experimental Toxicology*, *29*(10), 845–850. https://doi.org/10.1177/0960327110363441

Swerdlow, Joel, L. (2000). *Nature's Medicine: Plants that Heal: A chronicle of mankind's search for healing plants through the ages*. Washington, D.C.: National Geographic Society ; London : Hi Marketing.

Talalay, P., & Talalay, P. (2001). The importance of using scientific principles in the development of medicinal agents from plants. *Academic Medicine : Journal of the Association of American Medical Colleges*, *76*(3), 238–247. https://doi.org/10.1097/00001888-200103000-00010

Vagdatli, E., Gounari, E., Lazaridou, E., Katsibourlia, E., Tsikopoulou, F., & Labrianou, I. (2010). Platelet distribution width: A simple, practical and specific marker of activation of coagulation. *Hippokratia*, *14*(1), 28–32.

Whitton, P. A., Lau, A., Salisbury, A., Whitehouse, J., & Evans, C. S. (2003). Kava lactones and the kava-kava controversy. *Phytochemistry*, *64*(3), 673–679. https://doi.org/10.1016/S0031-9422(03)00381-9

Wiart, C. (2006). *Ethnopharmacology of Medicinal Plants Asia and the Pacific*. (J. Hackworth, Ed.). new jersey: human press.

Zhao, J., Davis, L. C., & Verpoorte, R. (2005). Elicitor signal transduction leading to production of plant secondary metabolites. *Biotechnology Advances*, *23*(4), 283–333. https://doi.org/10.1016/j.biotechadv.2005.01.003