EVALUATION OF NUTRIENT CONTENT, ANTIOXIDANT POTENTIAL AND ANTIMICROBIAL ACTIVITY OF SELECTED WILD EDIBLE FRUITS

A DISSERTATION SUBMITTED TO THE PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE MASTERS OF SCIENCE IN BOTANY

(PLANT SYSTEMATICS)

By

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To

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DECLARATION

The thesis entitled **"Evaluation of Nutrient Content, Antioxidant Potential, and Antimicrobial Activity of Selected Wild Edible Fruits"**, which is being submitted to the Department of Botany, Amrit Campus, Lainchaur, Kathmandu, Nepal for the award of the Master's Degree in Botany, is a research work carried out by me under the supervision of Dr. Giri Prasad Joshi and Dr. Lok Ranjan Bhatt.

This research is original and has not been submitted earlier in part or full in this or any other form to any University or Institute, here or elsewhere, for the award of any degree. Any literature, data or works done by others and cited within this thesis has been given due acknowledgement and listed in the reference section.

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सारांश

Wild edible fruits (WEFs) हरु नेपालको तराई देखी पहाडी भू–भागमा प्राकृतिक वातावरणमा प्रसस्त मात्रामा पाइने गर्दछन । यस्ता फलहरु पोषक पदार्थले भरिपूर्ण हुने गर्दछन । यस अध्ययनमा १२ ओटा त्यस्ता फलहरुलाई तिनको पोषकिय भिन्नता, phytochemical analysis, antioxidant क्षमता, र **antimicrobial activity को मूल्यांकन गरियो । यस** अध्ययनमा प्रयोग भएका फलहरुको संकलन मुख्यतः काठमाडौं उपत्यका बाट गरिएको थियो भने ३ ओटाको संकलन उपत्यका बाहिर बाट गरिएको थियो । फलहरुको नमूना परिक्षण नेपाल विज्ञान तथा प्रविधि प्रज्ञा प्रतिष्ठान (नास्ट) को खुमलटार स्थित प्रयोगशालामा **गरियो** । परिक्षण गरिएका प्रत्येक फलहरुमा निश्चित मात्रामा पोषण तत्वहरु भएको पाईयो । पोषण तत्वहरु मध्ये, प्रत्येक फलहरुमा moisture content प्रचुर मात्रामा थियो (५१.२०**±०.११ % देखि ९४.३१±०.४३ %**) । परिक्षण गरिएका wild fruits हरु मध्ये *Ardisia macrocarpa मा सर्वाधिक परिमाणमा* **total carbohydrates (१६.२±१.३२ %)** र protein (**०.७९±०.०४ %**) पाईयो । त्यस्तै *Phyllanthus emblica मा सर्वाधिक परिमाणमा* total phenolic content (**१३३.०६±०.३७** GAE mg/g) थियो भने, ***Ardisia macrocarpa* मा (२४५.३९±१.१६** QE mg/g) total flavonoid content, र *Phyllanthus acidus* (**२५.११±०.२७** mg/g) **मा** ascorbic acid थियो । *Phyllanthus emblica* र *Berberis asiatica का फलहरुको* DPPH scavenging assay उत्कृष्ट पाईयो, जसको IC_{50} value क्रमशः २.३५ μg/mL र १२.०३ μg/mL थियो । त्यसैगरि, सबै फलहरुको methanolic extract लाई सात ओटा रोगजनक सूक्ष्मजीव विरुद्ध antimicrobial activity का लागि परिक्षण गरियो । उक्त परिक्षणमा *Pyracantha crenulata*, *Phyllanthus emblica*, *Rubus paniculatus*, *Berberis asiatica*, र *Myrsine semiserrata* का extracts ले रोगजनक सूक्ष्मजीवको वृद्धि लाई सफलतापूर्वक रोकेको भेटियो । *Candida albicans* को वृद्धि लाई रोक्न सबै भन्दा बढि प्रभावकारी *Phyllanthus emblica (20 mm) को* extracts रहेको पाईयो भने दोस्रोमा *Rubus paniculatus* (17 mm), *र तेस्रामा Myrsine semiserrata* (17mm) रहेको पाईयो । यस अध्ययनको विश्लेषणले के देखाउदछ भने धेरै जसो wild edible fruits को पोषण तत्व र उपलब्ध प्राकृतिक रसायनको विविधताले गर्दा मानव स्वास्थ्यमा सकारात्मक प्रभाव पार्न सक्दछ, र जसलाई औषधि अथवा अन्य मूल्य–वर्धित उत्पादहरुको रुपमा प्रयोग गर्न सकिन्छ ।

ABSTRACT

Wild edible fruits (WEFs) are abundant in the natural environment and can be found in many parts of Nepal, varying in altitude from low to high. They are also highly rich in nutrients. The present study was carried out to evaluate the nutrient variability, phytochemical analysis, antioxidant potential, and antimicrobial activity of twelve different wild edible fruits that were mostly collected around the Kathmandu valley. Three of the fruits were collected from outside the valley. All the tests were conducted after drying the fresh, mature, and cleaned fruit samples at the Biological Resource Unit of the Nepal Academy of Science and Technology (NAST), Khumaltar. All the fruits were found to contain certain amounts of nutrients. Among the nutritional compositions, moisture content was abundant in every fruit, i.e., 51.20±0.11% to 94.31±0.43%. Present study reveals that *Ardisia macrocarpa* had the highest amount of total carbohydrates **(**16.2±1.32%) and protein (0.79±0.04%), among others. Phyto-chemicals such as total phenolic content were highest in *Phyllanthus emblica* (133.06 ±0.37 GAE mg/g), total flavonoid content in *Ardisia macrocarpa* **(**245.39±1.16 QE mg/g), and ascorbic acid in *Phyllanthus acidus* (25.11±0.27 mg/g). The DPPH scavenging assay of the fruit of *Phyllanthus emblica* and *Berberis asiatica* was found to be excellent, with IC_{50} values of 2. 35 µg/mL and 12.03 µg/mL, respectively. Similarly, the methanolic extract of all the fruits was tested for antimicrobial activity against the seven pathogens. The extracts of *Pyracantha crenulata*, *Phyllanthus emblica*, *Rubus paniculatus*, *Berberis asiatica*, and *Myrsine semiserrata* exhibited a significant zone of inhibition (ZOI) against all tested organisms. The highest zone of inhibition was recorded against *Candida albicans* for *Phyllanthus emblica* (20mm), *Rubus paniculatus* (17 mm), and *Myrsine semiserrata* (17mm) at 100 µg/mL of concentration. The present analysis indicates that several WEFs may be able to have positive health impacts due to their diverse nutritional components and natural phytochemicals, which can be used as medicine and other value-added products.

Keywords: *wild edible fruits, phytochemicals, antioxidant, DPPH, zone of inhibition*

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ABBREVIATIONS

$AlCl_3$:	Aluminum chloride
AOAC:	Association of Official Agricultural Chemists
ATCC:	American Type Culture Collection
BSA:	Bovine Serum Albimin
C:	chloramphenicol
DMSO:	Dimethyl Sulfoxide
DPPH:	2, 2- diphenyl- 1- picrylhydrazyl
FAO:	Food and Agriculture Organization
GAE:	Gallic acid equivalent
IC_{50}:	Half- maximal inhibitory concentration
IT:	Itraconazole
KATH:	National Herbarium and Plant Laboratories
MHA:	Muller- Hinton Agar
NA:	Nutrient Agar
NB:	Nutrient Broth
NPHL:	National Public Health Laboratory
QE:	Quercetin equivalent
rpm:	revolutions per minute
RSA:	Radical Scavenging Activity
TE:	tetracycline
TFC:	Total Flavonoid content
TPC:	Total Phenolic content
WEFs:	Wild edible fruits

CHAPTER I: INTRODUCTION

1.1 Background

There has been a close interaction between people and plants ever since the earliest days of human civilization due to the range of uses that people have made of wild plants (Rajbhandary *et al.,* 2020). Before the domestication of agricultural plants began, early humans consumed a wide variety of fruits, leaves, and roots that they had collected from the wild in order to survive. The species of edible plants that grow in wild rather than being tamed or farmed are known as wild food plants. In many rural parts of the world, these edible wild plants have played a vital role in providing impoverished communities with food and nutrition (Chakravarty *et al.,* 2016).

Naturally occurring, uncultivated plants that possess nutritional properties suitable for meeting dietary requirements are known as wild edible plants (WEPs). They provide a great supply of fiber, carbohydrates, vitamins, minerals, and other essential nutrients. They have, however, also been found to be important sources of phytochemicals in recent times, which either by themselves or in combination may be beneficial to human health (Bhutia *et al*., 2018). Through trial and error, primitive people picked various edible wild plants, domesticated them, and consumed them (Niveditha, 2017). Rural residents still rely on harvesting wild edible fruits for their nutritional needs, financial aspects, as well as for their primary medical care. These wild edible fruits have a substantial impact on rural people's livelihood and food security (Islam *et al*., 2019). Many ethnic people in Nepal, particularly those who live in rural areas, still gather fruits from wild plants, such as *Phyllanthus emblica* (Amla), *Ficus recemosa* (Dumri), *Rubus ellipticus* (Raspberry), and *Choerospondias axillaris* (Lapsi), which they then consume in various ways. Some of them even sell these fruits in local markets to supplement their income (Gautam *et al*., 2020).

The naturally occurring phytochemical substances present in different parts of medicinal plants (leaves, stem bark, fruits, and roots) possess the ability to act as a protective barrier against a range of harmful agents, such as viruses and bacteria. Secondary metabolites, which are naturally occurring plant compounds, are the byproducts of primary metabolism including lipids, amino acids, carbohydrates, and chlorophyll, among others. Secondary metabolites, which encompass a diverse array of substances such as glycosides, alkaloids, steroids, flavonoids, terpenoids, saponins, tannins, and phenolic compounds, are synthesized by them (Doss, 2009). Excellent antioxidant molecules are found in the secondary metabolites. Anti-inflammatory, anti-tumor, anti-atherosclerotic, antimutagenic, anticarcinogenic, antibacterial, antiviral, and antiparasitic qualities are found in large quantities in bioactive phytochemical substances (Rice-evans *et al*., 1995; Ashokkumar *et al*., 2008).

Antioxidants are chemicals that prevent or slow down oxidative damage to a target molecule. The capacity to contain free radicals is one of the primary features of antioxidants. Flavonoids, polyphenols, and phenolic acids are examples of antioxidant chemicals that scavenge free radicals like hydroperoxide, peroxide, or lipid peroxyl and prevent the oxidative damage that causes degenerative illnesses (Wu *et al*., 2011). Medicinal plants have long been thought to be superior antioxidants. Free radicals produced inside the body gets neutralized by the antioxidant such as glutathione or catalase but antioxidant are very less produced so the deficiency must be compensated by the use of natural exogenous antioxidents such as vitamin C, vitamin E, flavonoids, carotene and natural products in plants. Regarding antioxidant efficacy, dietary polyphenol obtained from plants shows remarkably more effect *in vitro* as compared to vitamin E or vitamin C. So plant based antioxidants have protective effect *in vivo* as well (Jayasri *et al*., 2009).

In Nepal, wild edible fruits could be the only source of edible fruits for the locals, particularly in the steep hilly regions where they may play a major role in their diet. Numerous crops are products of the domestication and subsequent enhancement of wild plants by humans. Even now, many more wild plants are used by Nepal's various ethnic groupings as food and beverages (Gautam *et al*., 2020). Among the most popular forest-derived products, wild edible fruits are significant sources of revenue, food, and medicine for those who used (Shrestha *et al*., 2020).

Wild edible plants give communities access to basic foods and sources of revenue, which is a significant contribution to food security and livelihood. Even in the modern world, these plants are especially important for impoverished, rural, and ethnic communities. These plants are still found in the wild, despite their significance for economic growth, livelihood, and food security. There are very few species that have been domesticated for use in medicine or religion. The public is still ignorant about the number, abundance, and availability of many of these plants because they are in their natural state, with the exception of a small number of researchers and the communities who have been using them on a regular basis for their livelihood and well-being.

1.2 Justification/ rationale

The most popular non-timber forest products are wild edible fruits, which are also significant sources of money, medicine, and nutrition for those who use them. Gathering wild fruits allows families to create jobs in rural areas and earn income through processing and value-adding.

Due to food insecurities and malnutrition, which affect roughly 821 million people worldwide, these problems continue to be a major global burden (FAO, 2019). Nearly 39% of mountain inhabitants in developing nations are undernourished, prone to food insecurity, and experience higher rates of infant and maternal mortality (FAO, 2015). Wild edible fruits available at the local level can play a significant role in food and nutritional security, particularly during food shortages and malnutrition, as they are free and easily available throughout the year. However, proper attention has not been paid to this opportunity in Nepal.

Additionally, the loss of nutrition in cultivated fruits has occurred due to several factors, both natural and human-induced. The main reasons behind the loss of nutrition in fruits are harvesting practices, storage conditions, processing and handling, transportation and shipping, post-harvest treatments and environmental factors. Due to the low nutrient content of their typical diets, people in rural areas often suffer from malnutrition. Therefore, researching the nutritional value of wild fruits can be quite helpful for replacing them in people's regular diets. Potential fruits for money, medicine, and food can be cultivated in similar situations.

1.3 Research Questions

a. Are there good phytochemical qualities in the wild edible fruits?
b. Which of the mentioned fruits exhibits a high overall phenolic and flavonoid content?
c. Which of the foregoing fruits contains a significant amount of antioxidants?
d. Are there antimicrobial properties in fruit methanolic extracts?

1.4 Objectives

General Objectives

The general objective of the present study was to evaluate the nutrient content, antioxidant potential and antimicrobial activity of selected wild edible fruits.

Specific Objectives

- To determine nutrient composition, such as carbohydrate, protein, fat, ash and moisture content
- To carry out the phytochemical screening of seasonal wild fruits using water and methanol solvent
- To determine total phenolic content (TPC) and total flavonoid content (TFC) from methanolic extracts
- To determine antioxidant activity of seasonal fruits by using DPPH free radical scavenging assay
- To determine the antimicrobial properties of dry methanolic extracts against ATCC cultures of common human pathogens

CHAPTER II: LITERATURE REVIEW

Numerous nutritious wild fruits, particularly those high in micronutrients (vitamins and minerals), can help meet the nutritional needs of humans and cattle. Studies have indicated that some wild fruits had a higher nutritional content compared to cultivated ones. They can offer a number of health advantages because they have been demonstrated to be rich in various vitamins, minerals, fibers, and polyphenols (Gautam *et al.*, 2020). Wild edible fruits have made a significant contribution to the food and nutritional needs of the poor in many rural areas of the world. In addition to having nutritional value, fruits' bioactive chemicals can help prevent chronic illnesses like diabetes, cardiovascular disease, neurological disorders, and malignancies of all kinds (Hegazy *et al.*, 2019).

2.1. Diversity of wild edible fruits in Nepal

Nepal's geographical difference in terms of altitude, ecology, and climate has resulted in a high level of biological variety, encompassing plant and animal diversity as well as socio-cultural diversity. According to Kharal and Dhungana (2018), there are 118 distinct ecosystem types in the country, with 112 of them being forest ecosystems. There are 11,971 flora recorded in Nepal, accounting for 3.2% of the total world (GoN, 2014). Nepal is rich in NTFPs species. In Nepal, there are 700 plant species that are used medicinally, 440 wild fruit species that are eaten, 30 spice species, and 71 species of non-timber forest products that provide fiber (Shrestha *et al.,* 2020).

In Bajracharya 1980a study "**Fruits sauvages comestibles de la vallée de Kathmandou**," he enumerated the wild fruits found in Kathmandu valley that are edible and included information on their native names, fruiting seasons, and edible components. A total of forty-five species from 37 genera were discovered. In Nepal, an estimated 199 wild edible fruit plant species have been found, representing 67 groups and 139 genera. People most frequently eat the fruits of tree species, shrubs, herbs, and climbers (Gautam *et al.,* 2020). Nonetheless, a study conducted in 2021 found that 256 wild edible fruits (WEFs), which belong to 68 families and 144 species, are consumed all throughout Nepal (Bhatt, 2021; Yangdon *et al.,* 2022).

2.2 Nutritional Assessment of wild edible fruits

Wild fruit trees are available seasonally everywhere and grow naturally in the wild without human assistance. The natives have utilized its leaves, bark, and root medicinally, but they eat the fruits as a nutrition supplement and as an ingredient in functional meals. Wild edible fruits are not only cheaper than cultivated fruits, but they also have higher levels of carbohydrates, protein, vitamins, phytochemicals, antioxidants, fiber, and minerals (Bayang *et al.*, 2021).

The "**Nutritive values of Nepalese edible wild fruits**" of 40 edible wild fruits were first examined by Bajracharya (1980b). The total dry matter, sugar, protein, total ash content, salt, potassium, magnesium, and calcium were among the findings. They collected fresh produce from all around the Kathmandu Valley. Similarly, comparing the nutritive values of ten edible wild fruits at their ripe and unripe stages, Bajracharya *et al.* (1982) studied the "**Comparison of nutritive values of some edible wild fruits at ripe and unripe stages"** from the Kathmandu valley. They did this by comparing the contents of dry matter, organic acid, total soluble sugar, crude protein, ash, sodium, potassium, calcium, and magnesium. The biochemical analysis's findings demonstrate that ripe and unripe fruits often have different chemical compositions.

Dhungel *et al.* (2017) studied the nutritional and physiochemical study of four selected species of *Berberis* fruit extract and found that *Berberis asiatica* were found superior with greater content of reducing sugar (2.36±0.02%), phosphorus (0.25±0.01%), whereas total protein was found greater in fruits of *B. angulosa* var. *fasciculata* (0.50±0.01%). Water soluble ash content was found greater in *B. angulosa* var. *fasciculata* (6.38%) and greater acid insoluble ash content was found in *B. angulosa* var. *angulosa* (2.95%).

2.3 Phytochemicals

Plants have their own defense and healing mechanisms against microbial injury as well as physical damage to any body part. Known also as phytonutrients, phytochemicals are the substances that are a part of this system. Although they have a range of unique structures, they are also known as specialized metabolites (Oz and Kafkas, 2017). Benzene rings containing one or more hydroxyl groups are a common component.

The ability of plants to synthesize active molecules, particularly non-nutritive chemical compounds, is nearly limitless. In a research by Mallikharjuna *et al.* (2007), a total of 12000 secondary metabolites were found, which represents less than 10% of the total. 10% of the almost 200,000 phytochemicals that were later found to exist were from grains, fruits, and vegetables (Sagdic and Tornuk, 2012). The most significant protective elements against illnesses were always thought to be vitamins and minerals, but new research indicates that phytochemicals may be far more potent than these nutrients. Phytochemicals offer a multitude of applications, such as antimicrobial, "antiviral," or "antibacterial," anti-inflammatory, and free radical scavenging properties (Oz and Kafkas, 2017).

Phytochemicals such as flavonoids, carotenoids, anthocyanins, and polyphenols were previously used to preserve food and food products and guard against microbiological degradation. As of right now, its applications include those for nutritional supplements, agrochemicals, flavors, fragrances, medications, coloring compounds, and bio-control agents (Negi, 2012). Phytochemicals can be broadly categorized into six main classes according to their chemical structure and properties. Alkaloids, lipids, phenolics, terpenoids, carbohydrates, and chemicals containing nitrogen are some of these classes (Huang *et al*., 2016). The main phytochemicals, which include glucosinolates, polyphenols, flavonoids, isoflavonoids, anthocyanidins, phytoestrogens, terpenoids, carotenoids, limonoids, phytosterols, and fiber, have been linked in numerous studies to possible health advantages.

2.4 Antioxidant activity

Free radicals and other reactive oxygen species are produced by the human body as byproducts of numerous physiological and enzymatic functions. The generation of reactive species (hydrogen peroxide, nitric oxide, peroxynitrile, and hypochlorous acid) and oxygen-related free radicals (superoxide and hydroxyl radicals) as well as from radon, cosmic radiation, electromagnetic radiation, xenobiotics, and pollution is primarily caused by aerobic metabolic processes. Antioxidants are substances that work to reduce, stop, or postpone the action of free radicals (Uttara *et al.*, 2009).

In addition to other test methods, the DPPH (2,2-Diphenyl-1-picrylhydrazyl) assay is one of the best analytical techniques for identifying antioxidant properties in plants, edible seeds, and essential oils because of its many advantages, which include low costs, ease of experimentation, consistency, acceptability for use at room temperature, and automation potential. This test is based on a neutralization process in which antioxidants donate electrons to neutralize free DPPH radicals. The term "IC_{50}," which refers to the effective antioxidant concentration needed to reduce the initial DPPH concentration by 50% is commonly used to describe this process (Munteanu and Apetrei, 2021).

Plants and their constituent parts—fruit leaves, bark, roots, and stems, for example—have been utilized for millennia to generate bioactive compounds with strong antioxidant properties, including flavonoids, phenolics, carotenoids, tannins, and vitamins (Lü *et al.,* 2010).

2.5 Antimicrobial activity

The world is very concerned about disease. One of the main causes of illnesses and fatalities is microbes. In addition to their nutritional worth, wild edible fruits are an excellent source of antioxidants and phytochemicals, which have antibacterial properties of their own. Fruit extracts, including peel, pulp, and juices, have been used to treat bacterial infections in a safer and more economical way due to the widespread availability of phytochemicals. Despite the fact that antibiotics are effective in preventing illness, the usage of natural phytochemicals is growing daily. Microorganisms are becoming more resistant to allopathic pharmaceuticals, despite the fact that new medications have been created using the plant and its components (Bernhoft, 2010). In response to microbial infection in vitro, plants have been discovered to produce flavones, flavonoids, and flavonols; these chemicals have been demonstrated to be effective antibacterial agents against a range of microbes (Bennet and Wallsgrove, 1994). Tannins have historically been used to treat wounds, catarrh, diarrhea, and hemorrhoids as well as to prevent oral inflammation (Ogunleye and Ibitoye, 2003).

2.6 Wild edible fruits

Naturally occurring fruit trees are available all year round and flourish without human assistance in their natural habitat. Although the fruits are eaten, the indigenous people have employed the leaves, bark, and root medicinally as an addition to nutritional supplements and functional meals.

2.6.1 *Pyracantha crenulata* (D.Don) M.Roem

Pyracantha crenulata (D.Don) M. Rosem fruit belongs to the family Rosaceae that is distributed in Nepal at an altitudinal range of 800-2800 m, in the West Himalaya, the East Himalaya, the Tibetan Plateau, Assam-Burma, and East Asia. It is called Ghangaru in Nepali. Its flowering period is March-May, whereas its fruiting period is July–September. Ripe red fruits are eaten fresh. Powdered dry fruits are taken in cases of bloody dysentery. Whereas, branches are used as walking sticks (Watson *et al*., 2012). During a thorough search for anti-inflammatory components in crude medications and plants, pyracrenic acid, a novel ingredient, was discovered in the bark of *Pyracantha crenulata* of the Rosaceae family by following its granulation-suppressive effect using the fertile egg method (Otsuka *et al.*, 1981).

2.6.2 *Phyllanthus acidus* (L.) Skeels

Phyllanthus, the largest genus of Phyllanthaceae, consists of *ca.* 1270 species that can be subdivided into 11 subgenera (Kathriarachchi *et al.*, 2006). *Phyllanthus acidus* is among them, commonly called Pate amala in Nepali, the Tahitian gooseberry tree, or the Otaheite gooseberry. This little deciduous tree can be found in damp coastal woodlands in tropical and subtropical regions, as well as disturbed areas.

P. acidus is known to have a prominent class of bioactive compounds, which include triterpenes, diterpenes, sesquiterpenes, and glycosides, according to phytochemical investigations conducted on its barks, leaves, roots, and fruits. *P. acidus* has been linked to a wide range of pharmacological activity, including as hepatoprotective and hypoglycemic effects in vivo, anti-oxidant, α-glucosidase inhibitory, anti-inflammatory, and antibacterial qualities in vitro (Tan *et al.*, 2020).

2.6.3 *Phyllanthus emblica* L.

Phyllanthus emblica, often known as Indian gooseberry or amla, is a medium height tree belonging to family Euphorbiaceae commonly found in Southeast asia. In Ayurved, fruit of amala is placed as most important medicinal part used in gastrointestinal as well as other many traditional medicinal compositions (Prasad and Srivastava, 2020).

Vitamin C (ascorbic acid) is a major compound found in Amla fruits. Furthermore, it contains a wide variety of physiologically active substances, such as minerals, vitamins, phenolic components (emblicanin A and B, ellagic acid, gallic acid, phyllembein, quercetin, terpenoids and flavonoids), and tannins with various effects (antioxidant, antipyretic, analgesic, hepatoprotective, memory enhancing, anticancer, antidiabetic, antidepressant, anti-ulcerogenic, insecticidal, larvicidal hypolipidaemic, chemoprotective, cytoprotective, antibacterial, antitussive, immunomodulatory, gastroprotective, and wound healing) (Khattak 2013; Khurana *et al.*, 2019). Khurana *et al.* (2019) has investigated the antimicrobial properties of *Phyllanthus emblica* fruit extracts are shown to have significant antibacterial properties against Gram-positive, Gram-negative, and resistant bacteria, with the maximum seen against *B. subtilis*, followed by *E. coli*, and the lowest against *S. paratyphi*. *Phyllanthus emblica* fruit extract has also been shown by Charoenteeraboon *et al*. (2010) to be a potent inhibitor of H_2O_2-induced cellular oxidative stress are a good antioxidant.

2.6.4 *Rubus paniculatus* Sm.

Rubus paniculatus Sm. is a shrub belonging to the family Rosaceae and commonly called 'Bhalu ainselu’ in Nepali. It is widely distributed in Nepal at an altitude of 1500–3200 m, in the West Himalaya, the East Himalaya, the Tibetan Plateau, Assam-Burma, and East Asia. It is commonly found in mixed forests on slopes, ravines, and stream sides. Ripe black fruits are edible.

The antioxidant capacity of *Rubus* fruits varies greatly based on genotype (Reyes-Carmona *et al*., 2005). This may be the cause of its historic use, in addition to treating anemia and hypertension, to treat moderate cardiac issues in isolated and tribal locations. Dried berry powder is also a component of traditional medicine for digestive issues. For skin conditions, dried leaf powder is combined with other ingredients to form a paste. Locals in isolated places used to grow this plant in their backyards for medical purposes, and it also served as a deterrent to wild animals for their grass shelters. Protein, carbohydrates, fat, sugar, energy value, and mineral contents like calcium, phosphorus, and nitrogen were all abundant in *R. paniculatus* (Rana *et al*., 2018).

2.6.5 *Morus nigra* L.

The *Morus nigra*, sometimes known as black mulberry, is a species of flowering plant in the Moraceae family that is native to Southwestern Asia. Its exact natural range is unknown as it has been cultivated for so long (Razdan and Thomas, 2021).

Mulberry fruits can be used to make syrup, jam, pulp, ice cream, vinegar, concentrate, and alcohol, among other things. In addition, mulberry leaves and other plant parts are utilized pharmaceutically across the world, particularly in China (Koyuncu, 2004). Mulberry fruits have various medicinal uses, including treating dysentery, acting as a laxative, odontalgic, anthelmintic, expectorant, hypoglycemic, and excitatory (Baytop, 1996). Many pharmacological characteristics, such as antinociceptive, anti-inflammatory, antimicrobial, anti-melanogenic, antidiabetic, anti-obesity, anti-hyperlipidemic, and anticancer activity, were demonstrated by *M. nigra*, particularly in relation to its leaf and fruit portions. The central nervous system, liver, kidney, gastrointestinal tract, and female reproductive system were also shown to be protected from when treated by *M. nigra*. The majority of these characteristics were linked to its antioxidant potential because it contained a lot of phytochemical components such anthocyanins, flavonoids, and polyphenols (Lim and Choi, 2019)

2.6.6 *Morus alba* L.

Mulberries, or *Morus alba* L., are a common species in the Moraceae family and are grown around the world, particularly in Southeast Asia, where they can withstand a range of temperatures and tropical conditions (Yuan and Zhao, 2017).

The fruit of *Morus alba*, also known as Kimbu, is rich in fiber, vitamins, minerals, lipids, and carbohydrates. The majority of vitamin C is found in all kinds of the *Morus* species, although *M. nigra* has the highest concentration. Due to the abundance of rich phytochemical and bioactive substances such as anthocyanins, rutin, quercetin, chlorogenic acid, and polysaccharides, mulberries as a whole have long been used as a functional food. The antibacterial, anti-hyperglycemic, anti-hyperlipidemic, anti-inflammatory, and anti-cancer effects of its extracts have been utilized to treat both acute and long-term conditions (Jan *et al*., 2021).

2.6.7 *Morus* sp.

Morus sp. is members of the Moraceae family, usually referred to as mulberries. Most people use its fruits as fresh fruit, jams, and juices. They have a sizable concentration of physiologically active components that may be connected to certain possible health-promoting pharmacological actions.

The fruit, wood, root, and leaves of the *Morus* plant contain a diverse array of phytochemicals that confer upon them a wide range of biological properties, including but not limited to antidiabetic, anti-obesity, anti-inflammatory, anti-cancer, antibacterial, antioxidant, and neuroprotective properties. Historically, the plant has been used to cure a wide range of ailments and to make a number of culinary products. Fruit-based products include wine, vinegar, jelly, jam, biscuits, bread, and squash, whereas leaf-based products include tea, toothpaste, pastries, and squash. However, the facility still lacks a unique character on an economic level (Dhiman *et al*., 2020).

2.6.8 *Smilax aspera* L.

The family Smilacaceae includes *Smilax aspera* L. It's known as "Kukurdaaino" in Nepali. This shrubby climber primarily consists of hooks and spines on its branches. It can be found between 1000 and 2000 meters above sea level. Parillin, potassium nitrate, and a heteroside are found in the roots and stems which utilized as a diuretic and as a medicinal alternative to Indian sarsaparilla (Nasir and Ali, 1970-89; Press *et al*., 2000). The *Smilax* genus has been shown to include trans-resveratrol, phenylpropanoid glycosides, lavonoid glycosides, anthocyanins, and steroidal saponins. It has been suggested that the primary active ingredients in these plants' therapeutic uses are steroidal saponins (Kostova *et al*., 2012).

2.6.9 *Ardisia macrocarpa* Wall.

The shrub *Ardisia macrocarpa* is a perennial of the Myrsinaceae family. It's known as "Damai phal" in Nepali. Around 500 species of *Ardisia* (Myrsinaceae) can be found worldwide in tropical and subtropical climates. Numerous *Ardisia* species have been used as culinary, medicinal, and ornamental plants. The use of *Ardisia* species is hindered by a lack of thorough investigation into their applications, despite their substantial supply of novel and physiologically potent phytochemical substances like bergenin and ardisin (Kobayashi and De Mejía, 2005). The bark and hardwood of *Ardisia macrocarpa* Wall contain rapanone (I), along with a leucoanthocyanidin, (−) 3, 4, 5, 7, 3′, 4′, 5′-heptahydroxyflavan (II) (Murthy *et al*., 1965)

2.6.10 *Averrhoa carambola* L.

The Oxalidaceae family includes the perennial tree *Averrhoa carambola* L., also known as star fruit or carambola. Although it is thought to be indigenous to Malaysia, the plant is really a tropical American one that was brought to Asia by Spanish galleons and is mostly grown in tropical and warm subtropical regions. The fruit has a significant market value and is grown and supplied specifically in southern China, northern South America, Southeast Asia, and India (Saikia *et al*., 2015; Leivas *et al*., 2016).

About 132 isolated compounds have been isolated from *A. carambola* thus far. Among these, it is believed that flavonoids, benzoquinone, and their glycosides are physiologically active substances that perform a range of biological tasks. Pharmacological studies have reported a wide range of bioactivities for crude extracts or monomeric compounds derived from *A. carambola*, including antioxidant, anti-hyperglycemic, anti-obesity, anti-hyperlipidemic, anti-tumor, anti-inflammatory, hepatoprotective, cardioprotective, anti-hypertensive, neuroprotective, and others. Consequently, *A. carambola* is a useful remedy in Chinese medicine that has the capacity to treat a variety of illnesses, including diabetes and conditions connected to it (Luan *et al*., 2021)

2.6.11 *Berberis asiatica* Roxb.ex DC.

Berberis asiatica also known as "Druhaldhi" and "Citra" in Hindi, “chutro” in Nepali, spinous, yellow bark herb of Berberidaceae family endemic to sub- Himalayan region of southern India, and Nepal. It has inhibitory impact on *Salmonella typhi in vitro* and antiamoebic action against *Entamoeba histolytica* (Rathi *et al*., 2013).

Alkaloids are the main bioactive chemical constituents of the *Berberis* species. Various pharma-cological activities such as antipyretic, antimicrobial, wound healing, antihyperglycemic, antioxidant, antitumor, anticancer, antioxidant, and several secondary metabolites (alkaloids, flavonoids, phenolic acids etc.) have been reported by Bhatt *et al*. (2018).

2.6.12 *Myrsine semiserrata* Wall. ex Roxb.

Myrsine semiserrata is a tree belonging to family Myrsinaceae. In Nepali, it is called as Kalikath. It is distributed in outer or sub –Himalayan tracts, India, Pakistan, Nepal and Burma. Its fruit is red in color becoming purble-black. Its seed is effective against Tapeworm (Bhattarai and Tamang, 2017). The aqueous paste of gum is given to cattle in relieving diarrhoea and dysentery (Gaur *et al*., 1992)

2.7 Economic importance of wild edible fruits

Wild fruits are woody species with a distinct taste, color, and flavor. Macro and micronutrients, as well as phytochemicals, are widely present in fruit's peel, pulp, and in seed. Wild fruits have been used in raw or processed form as diet supplements, financial support, and medicinal purposes, particularly by poor communities in village areas of numerous nations. Studies on wild fruits have increased since the 1990s because of their nutritional values and richness in natural antioxidants. In addition to fresh fruit consumption, they are used in the production of jams, jellies, and beverages such as wine, flavoring for ice cream, yoghurt, and so on. Furthermore, the use of natural extract antioxidant is superior to chemical preservers for food preservation. So there is huge potential for using wild fruits in rural areas to establish a mini-level cottage industry and also in the fields of feed, financial support, diet supplements, and food biotechnology (Mariod, 2019; Ercisli and Sagbas, 2017).

CHAPTER III: MATERIALS AND METHODS

3.1 Materials

A complete list of equipment's, materials, chemicals and reagent used in the whole study were enlisted in the Appendix A. Total 12 fruits sample were collected from their respective fruiting season. Nine fruit samples were collected from the different forests of Kathmandu Valley; one was collected from Jhapa District, one from Dolakha District, and one from Bardiya District. During the field visit, fruits were collected and packed in clean polythene bags, and data like altitude, latitude, and longitude were recorded there as well. Additionally, pictures have been taken, and branches with flowers and fruits have been compressed in newspaper for the herbarium.

Table 1: List of wild edible fruits with their geographical location

S.N	Plant Name	Locality	Altitude (m)	GPS Coordinates
1	*Pyracantha crenulata* (D. Don) M. Roem	Boson Village, Champa-devi	1512	27°39´08.64´´N, 85°15´58´´E
2	*Phyllanthus acidus* (L.) Skeels	Gulariya, Bardiya	140	28°11´15´´N, 85°27´15´´E
3	*Phyllanthus emblica* L.	Charikot, Dholakha	1567	27°39´59.8´´N, 86°3´1.48´´E
4	*Rubus paniculatus* Sm.	Champadevi, Kathmandu	1987	27°38´36.55´´N, 85°13´33.25´´E
5	*Morus nigra* L.	Khumaltar, Lalitpur	1282	27°39´24.80´´N, 85°19´39.78´´E
6	*Morus alba* L.	Khumaltar, Lalitpur	1282	27°39´24.89´´N, 85°19´39.85´´E
7	*Morus* sp.	Khumaltar, Lalitpur	1282	27°39´24.83´´N, 85°19´39.8´´E
8	*Smilax aspera* L.	Bhundole, Kathmandu	1911	27°38´10´´N, 85°14´59.98´´E
9	*Ardisia macrocarpa* Wall.	Bajrabarahi, Lalitpur	1482	27°36´19.07´´N, 85°19´50.07´´E
10	*Averrhoa carambola* L.	Buddhashanti, Jhapa	221	26°73´66.46´´N, 88°07´10.74´´E
11	*Berberis asiatica* Roxb. Ex DC.	Champadevi, Kathmandu	1987	27°38´36.45´´N, 85°13´33.35´´E
12	*Myrsine semiserrata* Wall.	Champadevi, Kathmandu	2002	27°38´27.58´´N, 85°15´31.25´´E

3.2 Lab Work

The research work was conducted in the Biological Resources Unit of Nepal Academy of Science and Technology (NAST) at Khumaltar, Lalitpur, Nepal, from December 2022 to September 2023.

3.3 Methods

3.3.1 Method of Sample Collection and Identification

Healthy and mature wild edible fruits were collected in a sterile plastic bag. The bag was labeled with the time, location, and date of sample collection before being transported to the laboratory in the ice box within 48 hours of sample collection and stored at 4°C in the refrigerator. Each fruit sample was chosen based on its potential for ethno-medical and commercial usage. All of the prepared herbarium was sent to the National Herbarium and Plant Laboratories (KATH), Godawari, Lalitpur, for official identification, and it was stored in the ASCOL Herbarium for further use.

3.3.2 Preparation of Samples

Healthy, harvested fruits were collected and cleaned with distilled water to remove the dust particles from the fruits. Then, pulp and seeds were separated with forceps and needles. After that, they were placed in a hot air oven set at 40°C for about a week to dry out and remove all of the moisture. Following drying, they were ground with a grinder to a fine powder and kept in plastic vials that were sealed tightly and labeled appropriately until they were needed again.

3.4 Nutritional Analysis

3.4.1 Total Carbohydrate Content

The clogg-anthrone method (Osborne & Voogt, 1978) was used to calculate the fruit sample's total carbohydrate content. A bluish green color was produced by hydrolyzing 1 gram of the sample in 13 mL of 52% perchloric acid, which then reduced the disaccharides, trisaccharides, and higher oligomers to their reducing sugar. This reaction was carried out in an acidic environment using an anthrone reagent. 500 µL of anthrone reagent was combined with an aliquot containing 100 µL. The absorbance of each microfuge tube was measured in the spectrophotometer at 630 nm after it had been incubated for 12 minutes in the dark. The results were expressed in mg/g of sample and the standard curve was generated using glucose (1 mg/mL).

3.4.2 Protein Content

A modified Bradford test was used to determine the protein content (Bradford, 1976). A conical flask containing 200 mg of powdered materials and 20 mL of distilled water was maintained at 50°C and 40 rpm for a full day (Bhusal *et al.*, 2020). After that, Whatman No. 1 filter paper was used to filter it, and the filtrate was utilized to analyze the protein. The microfuge tube was filled with the sample filtrate and a freshly made Bradford reagent in a 1:10 ratio. It was then placed in a dark room for no more than an hour. After that, 200 μL of each mixture were put into a 96-well plate, and the absorbance at 595 nm was calculated. The standard curve was created using various amounts of bovine serum albumin (1 mg/mL), and the results were expressed in mg/g of sample.

3.4.3 Fat content

Fat content was determined following Chew *et al*. (2011). The 5 g dry sample was weighed and kept in thimble paper, then placed into a Soxhlet extractor. Before the installation of the extractor, a dry, clean round-bottom (RB) flask was weighed, and 250 milliliters of petroleum ether solvent was added to the same RB flask. After that, the extraction unit was assembled and heated at 40–60 °C for the boiling of the solvent. The extraction process was continued for about 6 hours. Then the flask was removed from the extraction unit and heated in an oven at 70°C to remove the excess solvent. The flask was cooled in a desiccator, and weight was taken. Fat % was calculated using the formula:

$$\text{Crude Fat \%} = \frac{Weight\ of\ flask\ with\ extracted\ fat - weight\ of\ empty\ flask}{Weight\ of\ sample\ taken} \times 100$$

3.4.4 Ash Content

The ash content was determined following Chew *et al*. (2011). Five grams sample was placed in a crucible that had been cleaned and dried. The sample was then incinerated at 550°C for approximately 6 hours or until a yellowish or grayish ash appeared. The remaining inorganic stuff was weighed after being cooled in a desiccator and the biological matter was burned out.

3.4.5 Moisture content

The moisture content for fruit samples of each species was estimated as per Reeb & Milota (1999). Each sample was weighed five grams, put in a crucible that had been cleaned, and heated to 110˚C using a hot air oven. The crucible's final weight was recorded every two hours until it reached a consistent value. The moisture content was calculated using the formula below;

$$\text{Moisture }\% = \frac{Initial\ weight\ of\ crucible\ with\ sample - Final\ weight\ of\ crucible\ with\ sample}{Weight\ of\ sample\ taken} \times 100$$

3.5 Qualitative Phytochemical Analysis

Preparation of extract

For the methanolic extract, one gram of finely powdered fruit sample was placed into a conical flask, and twenty milliliters of methanol were then added. For 48 hours, the flask was maintained in a shaking incubator at 37°C and 40 rpm.

In a similar manner, 1 gram of powdered fruit samples was dissolved in 20 milliliters of water and incubated for 48 hours at 50°C and 50 rpm in a shaking incubator. It was filtered through glass vials containing Whatman No. 1 filter paper after 48 hours, and the filtrates were stored in a refrigerator for later use (Bhusal *et al*., 2020; Khalil *et al*., 2013).

The phytochemical tests were carried out by following standard protocols given by Dash *et al*. (2008), Khalil *et al*. (2013) and Kumar *et al*. (2007).

Different tests were performed for secondary metabolites.

a) Saponins: In a test tube, 2 mL of each solvent's extract and 6 mL of distilled water were added, and everything was well mixed. Saponins were present when persistent froth or bubbles appeared.

b) Tannins: In a test tube, 2 mL of extract from each solvent was added, along with 10% alcoholic ferric chloride. Tannins were present when a black or brownish-blue color formed.

c) Phenols: A test tube was filled with 2 milliliters of each solvent's extract and 2 milliliters of 5% aqueous ferric chloride. The phenols in a sample were detected by the formation of a blue color.

d) Cardiac glycosides: A test tube was filled with 1 mL of each solvent extract, 0.5 mL of glacial acetic acid, and 3 drops of 1% aqueous ferric chloride solution. The presence of cardiac glycosides was revealed by the formation of a brown ring at the contact.

f) Terpenoids: Each solvent's extract (1 mL) was added to the test tube along with 0.5 mL of chloroform and a few drops of strong sulfuric acid. The sample included terpenoids as evidenced by the formation of a reddish-brown precipitate.

g) Carbohydrates: One milliliter of each solvent's extract, a few drops of Molish's reagent, and one milliliter of concentrated sulfuric acid were added to the tubes' sides. After that, the mixture was left to stand for two to three minutes. The presence of carbohydrates in the sample was indicated by the formation of a red or dull violet color.

h) Steroids: After adding 2 mL of chloroform, 5 mL of aqueous plant crude extract, and conc. H_2SO_4 to the test tube, a red color formed in the lower layer of chloroform, indicating the presence of steroids.

3.6 Quantitative determination of Phytochemicals

Preparation of extract

A conical flask was filled with one gram of finely powdered fruit sample and 20 mL of methanol. The flask was incubated for 24 hours at 37°C and 40 rpm in a shaking incubator. The residue was again exposed to 20 mL of methanol for the following 24 hours after being filtered through Whatman No. 1 filter paper in glass vials after 24 hours. Both the filtrates were mixed in the same vials and kept in a refrigerator for further use (Bhusal *et al*., 2020). For the vitamin C content and carotenoids content, dry methanolic extract was used. For this, 5 grams of a finely powdered fruit sample were mixed in 60 mL of methanol at 37°C at 150 rpm for 48 hours. The extracts were filtered after 48 hours through a Whatmann No. 1 filter paper. The solvents were evaporated using a hot water bath set at 50°C (Ayoola *et al*., 2008).

3.6.1 Total phenolic content (TPC)

Total phenolic content was determined by using the Folin-Ciocalteau phenol reagent with slight modifications (Singleton & Rossi, 1965). After adding 150 µL of Folin-Ciocalteau phenol reagent and 50 µL of methanolic extract, the mixture was incubated for three minutes. Next, 150 µL of the usual saturated 10% sodium carbonate (Na_2CO_3) solution was added to the same microfuge tube, and 650 µL of distilled water was added to bring the level up to 1000 µL. For 90 minutes, the reaction was held in a dark room. After adding 200 µl of the reaction to the 96-well plate, the spectrophotometer was used to measure absorbance at 725 nm. The standard curve was prepared using 1 mg/mL of gallic acid, and the outcome was reported as mg of gallic acid equivalents (GAEs) per g of fruit extract.

3.6.2 Total Flavanoid content (TFC)

The aluminum chloride technique was used to determine the total flavonoid content (Chang *et al.,* 2002). The microfuge tube was filled with 500 µL of fruit extract and 500 µL of a 2% aluminum chloride solution. It was then left in a dark room for an hour. Methanol served as a stand-in. 200 µL of the reaction was applied to the clean 96-well plate after incubation, and the absorbance was measured at 515 nm. The standard was quercetin in methanol (1 mg/mL), and the findings were reported as mg of quercetin equivalents per g of extract.

3.6.3 Vitamin C content

The method developed by Klein and Perry (1982) was adapted to determine an amount of vitamin C. This involved dissolving 100 mg of dry methanolic extract for 45 minutes at room temperature in 10 mL of 1% meta-phosphoric acid. Whatman No. 1 filter paper was used to filter the mixture. 900 µL of 0.01% 2, 6-dichlorophenolindophenol (DCPIP) was added to 100 µL of filtrate and thoroughly mixed. After 30 minutes of incubation in a dark room, the absorbance at 515 nm was measured. L-ascorbic acid (1 mg/mL) was used to calculate the standard curve, and the result was expressed as mg of ascorbic acid per gram of extract.

3.6.4 Carotenoids Content

Modified protocols of Nagata and Yamashita (1992) were utilized to ascertain the level of β-carotene and lycopene. For one minute, 100 mg of dried methanolic extract was dissolved in 10 mL of acetone-hexane (4:6) combination. After giving the combinations a good shake, Whatman No. 1 filter paper was used to filter them. Next, the spectrophotometer was used to measure the absorbance at 453, 505, and 663 nm, respectively, after 200 µL of the sample had been put to the ELISA plate. The contents of β-carotene and lycopene were calculated according to the following equations:

Lycopene (mg/100 mL) = –0.0458A663 + 0.372A505 – 0.0806A453 and,

β- Carotene (mg/100mL) = 0.216A663 – 0.304A505 + 0.452A453.

3.7 Antioxidant Activity

The DPPH reagent was used to calculate the radical scavenging activity (Williams *et al*., 1995). A microfuge tube was filled with 900 µL of 0.1 mM diphenyl picrylhydrazyl (DPPH) reagent and 300 µL of methanolic extract. After 30 minutes of incubation in a dark room, the absorbance of the reaction was measured using a spectrophotometer set at 517 nm. As a positive control, ascorbic acid (1mg/mL) was used. Radical scavenging activity was determined by using formula.

$$\text{RSA\%} = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

The antioxidant activity of the methanolic extract was expressed as IC50 to inhibit the formation of DPPH radicals by 50%. IC_{50} was calculated by plotting the linear equation curve of RSA% vs. concentration.

3.8 Antimicrobial Activity

3.8.1 Crude extract preparation

Five grams of powdered dry fruits were dissolved in 60 mL of methanol at 150 rpm at 37˚C for 48 hours in a shaking incubator, and then filtered using Whatman filter paper no. 1. The filtrate was evaporated in a water bath heated to 50 °C. The dry extract was kept at 4 °C for later examination.

3.8.2 Preparation of stock/working solutions

A stock solution with a concentration of 100 mg/mL was made by dissolving 100 mg of the fruit sample's dry methanolic extract in 1 mL of DMSO. Likewise, concentrations of 25 mg/mL, 50 mg/mL, and 75 mg/mL were prepared and worked with in the antimicrobial test.

3.8.3 Collection of test organisms

The six ATCC strains of bacteria, namely *Escherichia coli* ATCC 25922, *Klebsiella pneumoniae* ATCC 700603, *Staphylococcus aureus* ATCC 25923, *Bacillus subtilis* ATCC 6051a, *Salmonella typhi* ATCC 19430, and *Enterococcus faecalis* ATCC 29212, as well as one strain of the fungus, *Candida albicans* ATCC 10231, were collected from the National Public Health Laboratory (NPHL), Teku, Kathmandu, Nepal. The bacteria were then sub-cultured on Nutrient Broth (NB) and further cultivated on Nutrient Agar petri plates.

3.8.4 Antimicrobial assay by agar well diffusion method

The preparation of sterile Muller Hinton Agar (MHA) measuring 9 cm was carried out in compliance with Appendix C's manufacturer's recommendations. An updated version of Nowak *et al*. (2022)'s approach was used to conduct an antimicrobial assay of fruit extract utilizing the agar-well diffusion method Using a sterile cotton swab, the freshly made bacterial inoculums were compared and matched with 0.5 MacFarland standards before being swabbed over Mueller-Hinton agar. Four wells were drilled into the medium containing inoculums and appropriately labeled using a sterile, 6 mm-diameter borer. A positive control consisting of specific antibiotics was positioned in the center of the plates, and 40 µL each of the test and negative control solutions (10% DMSO) were added to a test well. For positive control, suitable antibiotics were used. Then the plates were left at room temperature to allow the extracts to diffuse in the medium for about 30 minutes. After that, the plates were incubated at 37 °C for 24 hours. The zone of inhibition of the extract was measured in millimeters (mm) including the well in order to measure its antibacterial activity.

3.9 Data Analysis

Statistical analysis was performed using Microsoft Excel, and all data were collected in triplicates.

CHAPTER IV: RESULTS

Altogether, 12 wild edible fruit (WEF) samples were collected: 8 from Kathmandu district, 1 from Bardiya district, 1 from Dolakha district and 1 from Jhapa district (Table 2). The samples were examined for their nutrient composition, phytochemical status, antioxidant properties, and antimicrobial activity. The findings were interpreted as follows:

4.1 Nutritional Composition

Nutrient compositions like carbohydrate, protein, fat, ash, and moisture content were quantified (Table 2). Carbohydrate content was determined using a linear curve of standard glucose ranging from 10-500 µg/mL is y = 0.0008x + 0.1018, R^2 = 0.99. The minimum carbohydrate content was found in *P. crenulata* (2.4±0.24 %) and maximum in *A. macrocarpa,* i.e., 16.2±1.32 % of dry weight of the sample. Similarly, the protein content was determined from the calibration curve of bovine serum albumin (y = 0.0007x+0.1652, R^2 = 0.9886). Protein content varied from 0.79±0.04% (*A. macrocarpa*) to 0.04±0.01 % (*M. nigra*) of the sample.

Table 2: Carbohydrate, protein, fat, ash and moisture content of wild edible fruits

Samples	Carbohydr-ates (%)	Proteins (%)	Fat (%)	Ash con-tent (%)	Moisture content (%)
Pyracantha crenulata	2.4±0.24	0.66 ± 0.01	0.84±0.69	0.80±0.56	84.12±0.65
Phyllanthus acidus	8.75±0.68	ND	0.62±0.72	0.53±0.21	89.34±0.32
Phyllanthus emblica	4.49±0.4	0.11±0.02	3.32± 0.22	0.61±0.12	85.23±0.21
Rubus paniculatus	10.64±0.13	0.08±0.04	10.83±0.11	0.36±0.11	88.45±0.43
Morus nigra	9.76±1.44	0.04±0.01	7.84±0.12	1.27±0.09	80.92±0.09
Morus alba	15.33±1.05	0.27±0.11	7.08±0.43	1.01±0.05	77.72±0.31
Morus sp.	7.58±1.18	0.67±0.02	3.46±1.09	1.11±0.17	80.72±0.28
Smilax aspera	6.71±0.25	0.36 ± 0	3.41±0.69	1.18±0.09	71.64±0.45
Ardisia macrocarpa	16.2±1.32	0.79±0.04	1.27±1.21	1.13±0.09	70.90±0.54
Averrhoa carambola	10.17±0.59	0.38 ± 0.01	2.49±0.75	1.89±0.11	94.31±0.43
Berberis asiatica	8.14±0.29	0.09±0.03	7.68±0.55	1.08±0.20	74.47±0.59
Myrsine semiserrata	3.13±0.28	0.08±0.01	7.64±0.54	1.90±0.33	52.51±0.10

Likewise, the highest amount of fat was determined in *R. paniculatus* (10.83±0.11%) whereas the lowest was found in *P. acidus* (0.62±0.72%). The ash content varied from 0.36±0.11% (*R. paniculatus*) to 1.90±0.33% (*M. semiserrata*). Furthermore, maximum amount of moisture was found in *A. carambola* (94.31±0.43%) and lowest in *M, semiserrata* (52.51±0.10%).

4.2 Preliminary Qualitative Phytochemical Analysis

As a preliminary test, the fruits were screened for terpenoids, phenols, saponins, carbohydrates, tannins, cardiac glycosides, and steroids using phytochemical analysis. According to Table 3, the methanolic extract of the largest amount of wild edible fruits included carbohydrates, cardiac glycosides, and terpenoids. But only the fruit sample from *A. macrocarpa* contained saponin.

Similarly, in the case of water extract, phytochemicals were comparatively lower than those in the methanolic extract, so further quantitative phytochemicals were extracted from the methanolic extract. However, saponins and cardiac glycosides were present more in the water extract.

Table 3: Preliminary Qualitative Phytochemical Analysis of Methanolic and Water Extract of Wild Edible Fruits.

Samples	**Sap.**		**Phe.**		**Ter.**		**Car.**		**Tan.**		**Cgl.**		**Ste.**	
	M	**W**	**M**	**W**	**M**	**W**	**M**	**W**	**M**	**W**	**M**	**W**	**M**	**W**
Pyracantha crenulata	-	+	-	-	+	-	++	+	++	+	+	-	+	-
Phyllanthus acidus	-	-	+	+	+	+	++	+	-	-	+	+	-	-
Phyllanthus emblica	-	-	++	++	++	-	++	+	++	++	+	+	-	-
Rubus paniculatus	-	+	++	+	++	-	++	+	++	+	+	+	++	+
Morus nigra	-	-	+	+	++	-	++	+	+	+	-	+	+	+
Morus alba	-	-	+	+	++	-	++	+	-	-	-	+	-	-
Morus sp	-	-	+	+	++	-	++	+	-	-	+	+	-	-
Smilax aspera	-	+	-	-	+	-	++	+	+	+	+	+	+	-

Ardisia macrocarpa	+	+	+	-	++	+	++	+	+	-	+	+	+	-
Averrhoa carambola	-	+	-	-	+	-	++	+	+	+	+	-	-	-
Berberis asiatica	-	-	++	+	+	+	++	+	+	-	+	+	+	-
Myrsine semiserrata	-	-	++	-	+	-	++	+	+	-	+	+	-	-

Legend: M **=** Methanolic Extract and W= Water Extract

Sap.: Saponins; Phe.: Phenols; Ter.: Terpenoids; Car.: Carbohydrates; Tan.: Tannins; Cgl.: Cardiac Glycosides; Ste.: Steroids ,

++: highly present, +: moderately present, - : low

4.3 Quantitative determination of phytochemicals

The methanolic extract of wild edible fruits was used to quantitatively determine phytochemicals such as total phenolic content (TPC), total flavonoid content (TFC), ascorbic acid (Vitamin C), β-carotene, and lycopene (Table 4). Total phenolic content (TPC) of wild edible fruits was determined with a linear curve of standard gallic acid (y = 0.002x + 0.0596, R^2 = 0.992) was obtained. Samples ranged in TPC from 3.45 ±0.11 to 133.06 ±0.37 mg GAE/g of dry extract. *P. emblica* had the highest TPC content, whilst *P. acidus* had the lowest. The total flavonoid content (TFC) of several fruit extracts varied in a manner akin to this. Total flavonoid content was estimated, where quercetin was used as a standard with a linear equation (y = 0.0003x+0.047, R^2 = 0.9988). The highest amount of TFC was found in *A. macrocarpa* (245.39±1.16 mg QE/g of dry extract), and the lowest was found in *P. crenulata* (35.67±1.54 mg QE/g of dry extract).

Table 4: TPC, TFC, Vit-C, β-carotene and lycopene content of wild edible fruits

Samples	TPC (mg GAE/g)	TFC (mg QE/g)	Vitamin C (mg/g)	β-carotene (mg/g)	lycopene (mg/g)
Pyracantha crenulata	37.65 ±0.66	35.67 ±1.54	4.09±0.34	0.02±0	0.01±0
Phyllanthus acidus	3.45 ±0.11	60.6±0.13	25.11±0.27	0.05±0	0.03±0
Phyllanthus emblica	133.06 ±0.37	62.57±0.39	14.75±0.04	0.02±0	0.01±0
Rubus paniculatus	22.13 ±1.02	197.64 ±4.47	19.48±0.28	0.04±0	0.02±0
Morus nigra	9.82 ±0.09	118.8±0.33	20±0.55	0.02±0	0.01±0
Morus alba	4.96 ±0.95	75.64±0.49	22±0.78	0.03±0	0.02±0
Morus sp.	5.03 ±0.33	69.39 ±1.41	24.82±0.17	0.02±0	0.01±0
Smilax aspera	16.63 ±0.45	60.01 ±1.76	10.29±0.58	0.02±0	0.01±0
Ardisia macrocarpa	15.26 ±1.23	245.39±1.16	13.94±0.53	0.02±0	0.01±0
Averrhoa carambola	18.59 ±0.19	39.81±0.13	10.67±0.18	0.02±0	0.01±0
Berberis asiatica	39.05 ±0.39	86.36 ±3.96	15.41±0.1	0.03±0	0.01±0
Myrsine semiserrata	12.32 ±0.67	113.37 ±2.67	5.61±0.11	0.17±0.01	0.06±0.01

Likewise, Vitamin-C was determined using ascorbic acid standard with a linear curve of $y = -0.0019x+1.3856$, $R^2 = 0.9972$. It varied from 4.09±0.34 mg/g of dry extract (*P. crenulata*) to 25.11±0.27 mg/g of dry extract (*P. acidus*).Similarly, β-carotene and lycopene were determined from a dry methanolic extract. In almost every fruit sample, β-carotene and lycopene were only marginally detectable. The ranges for β-carotene and lycopene in a dry extract are 0.02±0 to 0.17±0.01 mg/g and 0.01±0 to 0.06±0.01, respectively.

4.5 Determination of Antioxidant Activity

IC_{50} was also calculated from the standard curve of concentration *vs.* RSA%. Methanolic extracts of fruit *P. emblica* and *B. asiatica* showed greater antioxidant properties with IC_{50} values of 2.35µg/mL and 12.03µg/mL, respectively, as compared with ascorbic acid, which had an IC_{50} value of 21.30µg/mL, whereas the least antioxidant property was found in *Morus* sp. 95.08µg/mL, as shown in Figure 1.

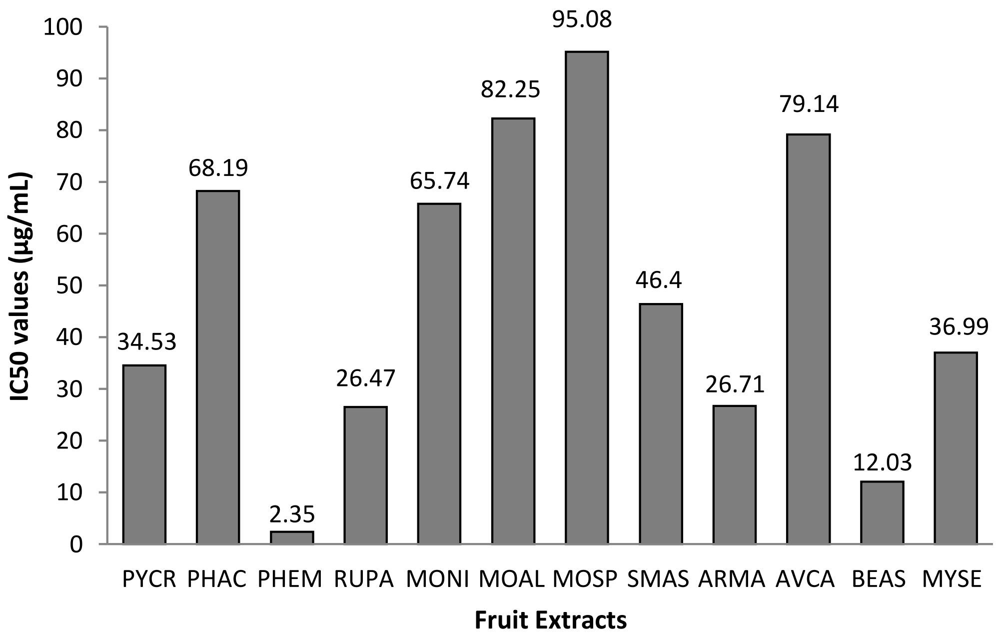

Figure 1: IC_{50} values of different fruits samples

Legend: PYCR- *Pyracantha crenulata*, PHAC- *Phyllanthus acidus*, PHEM- *Phyllanthus emblica*, RUPA- *Rubus paniculatus*, MONI-*Morus nigra*, MOAL- *Morus alba*, MOSP- *Morus* sp., SMAS- *Smilax aspera*, ARMA- *Ardisia macrocarpa*, AVCA- *Averrhoa carambola*, BEAS-*Berberis asiatica*, MYSE-*Myrsine semiserrata*

4.6 Antimicrobial Activity of Fruit Extract

Methanolic extracts of fruits were tested for their antimicrobial activity (Table 5). The methanolic extracts of *P. crenulata, P. emblica, B. asiatica, R. paniculatus,* and *M. semiserrata* exhibited significant antibacterial activity against all three gram-negative bacteria, while *A. macrocarpa, S. aspera, M. alba, M. nigra, Morus* sp., and *P. acidus* did not show any antibacterial activity against all three gram-negative bacteria. Likewise, *A. carambola* exhibited a zone of inhibition only against *Salmonella typhi*. Dimethyl sulfoxide (DMSO) was used as a negative control. Tetracycline (30 µg) was used for *Escherichia coli*, *Klebsiella pneumoniae, Enterococcus faecalis*; chloramphenicol (30 µg) for *Bacillus subtilis, Staphylococcus aureus*, *Salmonella typhi* and itraconazole (10 µg) for *Candida albicans* were used as positive controls in the tested organisms.

Table 5: Zone of inhibition (in mm) of fruit extracts against gram negative bacteria

Samples	Pathogens	Zone of inhibition (in mm)				
		100 mg/mL	75 mg/mL	50 mg/mL	25 mg/mL	Positive controls
Pyracantha crenulata	*E. coli*	14	14	13	11	24
	S. typhi	9	7	7	0	25
	K. pneumoniae	8	8	8	0	26
Phyllanthus emblica	*E. coli*	9	7	0	0	24
	S. typhi	10	9	8	0	25
	K. pneumoniae	10	9	8	0	26
Rubus paniculatus	*E. coli*	13	11	11	10	24
	S. typhi	15	13	10	8	25
	K. pneumoniae	13	12	10	8	27
Averrhoa carambola	*E. coli*	0	0	0	0	24
	S. typhi	21	19	0	0	25
	K. pneumoniae	0	0	0	0	27
Berberis asiatica	*E. coli*	8	0	0	0	25
	S. typhi	9	8	0	0	26
	K. pneumoniae	9	8	8	8	26
Myrsine semiserrata	*E. coli*	15	14	14	13	24
	S. typhi	11	9	8	0	25
	K. pneumoniae	13	11	11	8	26

Note: *P. acidus*, *M. alba*, *M. nigra*, *Morus* sp. *S. aspera* and *A. macrocarpa* does not show any zone of inhibition against the above gram negative pathogens.

Similarly, gram-positive bacteria and yeast (*Candida albicans*) concentrations of 25, 50, 75, and 100 mg/mL were tested for antimicrobial activity (Table 6). The methanolic extracts *of P. crenulata, P. emblica, R. paniculatus, B. asiatica* and *M. semiserrata* exhibited the significant antimicrobial activity against all gram positive bacteria and *C. albicans*. *P. acidus* extract showed zone of inhibition (ZOI) against only against *S. aureus. A. carambola* extract showed zone of inhibition (ZOI) against only *C. albicans*. Likewise, *M. nigra* and *M. alba* fruit extract showed zone of inhibition against only in *E. faecalis* whereas *A. macrocarpa, S. aspera,* and *Morus* sp. extracts did not exhibit any antimicrobial activity against gram positive bacteria and *C. albicans*.

Table 6: Zone of inhibition (in mm) of fruit extracts against gram positive bacteria and *C. albicans*

Samples	Pathogens	Zone of inhibition (in mm)				
		100 mg/mL	75 mg/mL	50 mg/mL	25 mg/mL	Positive Controls
Pyracantha crenulata	*E. faecalis*	9	9	8	8	24
	B. subtilis	12	11	9	7	27
	S. aureus	13	10	10	8	28
	C. albicans	14	13	12	11	28
Phyllanthus acidus	*S. aureus*	12	10	9	0	29
Phyllanthus emblica	*E. faecalis*	10	0	0	0	24
	B. subtilis	21	16	14	0	27
	S. aureus	15	15	12	10	29
	C. albicans	20	19	16	15	27
Rubus paniculatus	*E. faecalis*	12	11	9	9	24
	B. subtilis	11	11	11	9	28
	S. aureus	16	15	12	9	28
	C. albicans	17	15	14	12	26
Morus nigra	*E. faecalis*	19	14	9	0	24
Morus alba	*E. faecalis*	13	0	0	0	25
Ardisia macrocarpa	*E. faecalis*	9	0	0	0	24
Berberis asiatica	*E. faecalis*	9	8	8	7	25
	B. subtilis	9	9	8	0	27
	S. aureus	11	9	8	0	29
	C. albicans	7	7	0	0	26
Myrsine semiserrata	*E. faecalis*	12	11	11	10	25
	B. subtilis	14	12	9	8	27
	S. aureus	12	11	9	8	29
	C. albicans	17	17	16	14	26

Note: *A. carambola*, *Morus* sp. and *S. aspera* does not show any zone of inhibition against the above gram positive pathogens and *C. albicans*.

CHAPTER V: DISCUSSION

Wild edible fruits are highly appreciated crops due to their distinct flavors, textures, and colors. Wild edible fruits have recently been demonstrated to have considerable health advantages due to their high antioxidant content, vitamins and minerals, fiber, and folic acid. In addition to fresh consumption, they are frequently observed in beverages, ice cream, yoghurt, jams, jellies, and a variety of other food products. Rural and tribal groups depend heavily on a variety of wild edible fruits for their livelihood (Ercisli and Sagbas, 2017). However, human activities, such as changes in land use, including the expansion of the agricultural sector, leading to deforestation, have also resulted in the loss of wild edible fruit diversity (Beckmann *et al.,* 2019).

In the current research, wild edible fruits with nutritional and medicinal qualities were selected for the evaluation. The solvent used for extraction is methanol because of its high polarity, which could produce a high extraction yield. Lower-molecular-weight polyphenols have generally been found to be easier to extract with methanol (Mokhtarpour *et al*., 2014). However, climatic conditions, temperature, parts of the plant used, extraction time, and extraction procedure play an important role in isolating bioactive compounds that have pharmacological activity (John *et al*., 2006).

As a part of nutritional variability, parameters like carbohydrate content, protein content, fat, moisture, and ash were investigated in the study. The nutrition composition of selected wild edible fruits revealed that the moisture content was higher than other parameters, followed by carbohydrate content. The fruit of *A. carambola* had the highest moisture content, i.e., 94.31±0.43%, followed by *P. acidus* at 89.34±0.32% and *R. paniculatus* at 88.45±0.43%, respectively. According to Edem et *al*. (2008), the moisture content of ripe and unripe fruit of *A. carambola* was 85.1% and 92.5% respectively, whereas the moisture content of *P. acidus* fruit reported by Shilali (2015) was 91.7% and by Mahapatra *et al*. (2012) was 86.7%, which was nearly equal with the results of this study. Similarly, the moisture content of *R. paniculatus* reported by Rana *et al*. (2018) was 84.0% which was less than the present study. Higher moisture content suggests shorter storability if not treated within an appropriate time frame. The reason for its high moisture content could be attributed to an increase in the percentage of juice in its fruits and a decrease in the percentage of total dissolved solids, which indicates that it is one of the types appropriate for manufacturing juices and jams (Gerasopoulos and Stavroulakis, 1997). The reason for the slight difference in the moisture value might be the fruit maturity time, harvesting time, storage duration, soil moisture and humidity. Similarly, *M. semiserrata* had the least moisture value, i.e., 52.51±0.10%. *M. semiserrata* fruit typically has a lower moisture content compared to other fruits due to its thicker skin (pericarp), which reduces pulp volume, and its ecological adaptability in arid or semiarid climates where water availability may be limited. As a result, this species evolved mechanisms to conserve water, including producing fruits with lower moisture content.

Carbohydrates are the most commonly found macronutrient in wild fruits, and they can be categorized as reducing or non-reducing sugars. They are a major energy source, which determines the quality of fruit. Beside moisture, it covers the highest percentage of nutrient composition. In this study, *A. macrocarpa* has the highest carbohydrate content, i.e., 16.2±1.32% followed by the *M. alba* (15.33±1.05 %), while *P. crenulata* has the lowest, i.e., 2.4±0.24%. Mahapatra *et al*. (2012) reported in their study that the carbohydrate content was found to range from 3.1% to 19.01% while examining the 15 wild edible fruits of Indian forests. According to Imran *et al*. (2010), total carbohydrates of *M. alba* and *M. nigra* were 14.21% and 13.83%, respectively, which was quite similar to our study. A study by Singh *et al*. (2018) reported the carbohydrate content of *P. crenulata* ranges from 0.96 to 5.90%, and the carbohydrate value of *P. crenulata* in this study fell within this range. Similarly, the previous study done by Mahapatra *et al*. (2012) reported the carbohydrate content of *P. acidus* was 4.81±0.54% which was lower than the present study i.e., 8.75±0.68%. Also, according to Bhaskar and Shantaram (2013), the carbohydrate content of *A. carambola* was 9.78 ± 0.1%, which is similar to our finding, i.e., 10.17 ± 0.59%. The variation in carbohydrate content might be observed due to the maturity of fruits, moisture level, geographical as well as climatic variation, and applied extraction methods used during analysis (Aular *et al*., 2017). Sunlight and wind contribute greatly to the desiccation of fruit, which can have a considerable impact on the carbohydrate content (Ruiz-Rodríguez *et al.*, 2011)

After carbohydrates, protein is the second major macronutrient in fruit and is available in soluble form. It serves a variety of biological purposes in the body. In terms of both protein and fat composition, fruits are essentially cheap sources (Dhungel *et al*., 2017). In this study, total protein content in wild edible fruits ranges from 0.04±0.01% to 0.79±0.04%. According to the study done by Saklani and Chandra (2014) and Rana *et al*. (2018), the protein content of *P. crenulata* was 5.13 ± 0.04% and 8.47 % respectively which was higher than the current research, i.e., 0.66 ± 0.01%. Rana *et al*. (2018) reported that the protein content in *R. paniculatus* was 8.77%, which was higher than the present study. Similarly, protein content of *A. carambola* was 0.53 ± 0.04% (Bhaskar and Shantaram, 2013) and 4.0-7.9% (Edem *et al*., 2008) which was little more than current research i.e., 0.38 ± 0.01%. The difference in protein content might be due to the different solvent used, harvesting season and geographical area (Hegazy *et al*., 2019).

In a similar vein, Soxhlet extraction with petroleum ether solvent was used in this study to ascertain the fat content of the chosen fruit sample. Fatty acids like linoleic and linolenic acid, which are abundant in plants and necessary for embryonic development and normal brain function, are also plentiful in plants. Fruit oil contains ß-asarone, a known sedative and anticancer agent, and trans-methyl isoeugenol, a stimulant of oviposition (Shrestha *et al*., 2021). In this study, the fat percentage of wild edible fruits ranges from 0.62±0.72% to 12.36±0.34%. The lowest fat percentage was 0.62±0.72% for *P. acidus* which is more or less similar to finding of Shilali (2015), i.e., 0.52%. However, the fat percentage in *P. crenulata* in our study was 0.84±0.69%, which is in between the values reported by Rana *et al*. (2018) i.e. 0.54% and Saklani and Chandra (2014) i.e. 1.00±0.25%. A study by Edem *et al*. (2008) reported that the fat percentage of *A. carambola* was 3.5%, which was quite more than the current study, i.e., 2.49±0.75%. The recorded fat percentage of *M. alba* and *M. nigra* from the current study was 7.08±0.43% and 7.84±0.12%, respectively, which is higher than the result reported by Ercisli and Orhan (2007) and Hepsağ *et al*. (2016), i.e., 1.10% and 0.95%; and 0.99% and 0.87%, respectively, which was due to the different solvent used (n-hexane). Koyuncu *et al*. (2014) reported 3.15% to 7.26% of fat percentage for eight genotypes of *M. nigra*, which is very similar to the current study using the same solvent. The differences in fat percentage might be attributed to changes in sample and processing procedures, geo-climatic conditions, plant age, fruit maturity, species variances, and harvesting environment (Ercisli and Orhan, 2007).

Ash is the powdery residue that remains after burning biological products. The amount and content of ash in a food product are determined by the nature of the food burned and the manner of ashing (Pomeranz and Meloan, 1994). The mineral strength of *R. paniculatus* and *M. semiserrata* was assessed by their ash content, which ranged from 0.36±0.11% in *R. paniculatus* to 1.90±0.33% in *M. semiserrata* in the current study. Consuming these species can significantly reduce micronutrient deficiencies that negatively impact human health. According to the data reported by Gungor and Sengul (2008) and Abbasi *et al.* (2016), the ash content of *M. alba* ranges from 2.20±0.00% to 2.65±0.07% *and* 4.41±1.48%, respectively, which is high compared to the current study, i.e., 1.01±0.05%. Similarly, Koyuncu *et al.* (2014) reported 0.36%–0.12% of the ash percentage of eight different genotypes of *M. nigra,* which is less than the current study, i.e., 1.27±0.09%. These differences can be attributed to ecological factors and harvesting time. Similarly, 1.89±0.11% of ash was determined in *A. carambola,* which was more than that reported by Bhaskar and Shantaram (2013), i.e., 0.34 ± 0.02%, but less than that reported by Edem *et al*. (2008), i.e., 3.5%. Likewise, the ash content of *P. crenulata* was 0.80±0.56%, which is somewhat similar to the data reported by Dincer and Temiz (2023), i.e., 0.85%, but less than the data reported by Tewari *et al*. (2020) from Uttarakhand, i.e., 2.98 ± 0.78%. Khattak's (2015) findings imply that geographical distributions influence phytochemical levels and, consequently, biological activity. These variances must be considered when using raw plant materials in industrial applications or traditional remedies.

For the preliminary qualitative screening of the phytochemicals, metanolic and water extracts were prepared for the test. The majority of fruits in methanolic extract contain phenols, terpenoids, carbohydrates, cardiac glycosides, tannins, and steroids, which suggests the presence of several secondary metabolites and the potential medical use of those fruits. But saponins were present only in *A. macrocarpa.* This study revealed that the methanolic extract contains more phytochemicals compared to the water extract. This may be due to the polarity of methanol (Mokhtarpour *et al*., 2014). However, saponins were present in a larger number of samples from water extract (*P. crenulata, R. paniculatus, S. aspera, A. macrocarpa,* and *A. carambola) Saponins* have amphiphilic properties, meaning they have both hydrophilic (water-attracting) and hydrophobic (water-repelling) parts in their molecular structure. Water can penetrate the plant cells and solubilize the hydrophilic portions of saponin molecules effectively. Additionally, some saponins may form stable complexes with water molecules, facilitating their extraction, whereas methanol tends to dissolve primarily hydrophobic compounds rather than hydrophilic ones and methanol may not interact as effectively with the hydrophilic parts, leading to lower extraction yields (Mugford and Osbourn, 2013).

In parallel, phytochemicals were also determined in the current study. Phytochemicals like total phenolic content, total flavonoid content, ascorbic acid, beta-carotene, and lycopene had been evaluated in the methanolic extract. The presence of phytochemicals (secondary metabolites) determines the texture, flavor, and nutritional aids of fruit, which have numerous health benefits for living beings, including antioxidant, antiallergic, and anti-inflammatory properties, as well as the potential to reduce cancer cell and myocardial stress (Gündeşli *et al*., 2019). The amount of total phenolic content (TPC) in wild edible fruits had significant variation, ranging from 3.45 ±0.11 mg GAE/g to 133.06 ±0.37 mg GAE/g. *P. emblica* was observed to have the highest TPC value compared to other samples, i.e., 133.06 ±0.37 mg GAE/g, followed by *B. asiatica* (39.05 ±0.39 mg GAE/g) and *P. crenulata* (37.65 ±0.66 mg GAE/g), whereas *P. acidus* has the least TPC (3.45 ±0.11 mg GAE/g). The TPC value of *B. asiatica* fruit was measured 53±1.34mg GAE/g reported by Gaur *et al*. (2017) and 50.27 mg GAE/g reported by Dhungel *et al*. (2016) which was greater than the current study i.e. 39.05±0.39mg GAE/g. The TPC value of *P. crenulata* recorded by Guglani *et al*. (2021) was 39.01 mg/g, which was quite similar to the current research. The total phenolic content of *M. alba* and *M. nigra* was 4.96 ±0.95 and 9.82 ±0.09 mg GAE/g in this study. Earlier, Ercisli & Orhan, (2007) reported was 1.81 and 14.22 mg GAE/g respectively from deionized water extracts. In addition, Lin & Tang (2007) found that the total phenol content in *M. alba* was 15.159 ± 5.7 mg GAE/ g of fresh matter. Many factors, including the degree of fruit maturity at harvest, genetic variations, and environmental conditions throughout fruit growth, influence the variance of phenolic chemicals in the fruits (Zadernowski *et al*., 2005).

In the same way, the total flavonoid content (TFC) of wild edible fruits ranged from 35.67 ±1.54 to 245.39 ±1.16 mg QE/g, with *A. macrocarpa* having the highest TFC level, followed by *R. paniculatus* 197.64 ±4.47 mg QE/g, and *P. crenulata* (35.67 ±1.54g QE/g) had the lowest. The TFC value of *M. nigra* and *M. alba* was 118.8±0.33 mg QE/g and 75.64±0.49 mg QE/g in this study, whereas previously Tewari *et al*. (2023) reported 275.55±11.88 mg QE/100g and 20.18±0.47 mg QE/100g from ethanol extract and Abbasi *et al*. (2016) reported 95.8 ± 10.7mg RE/100g and 27.5 ±

2.41 mg RE/100g from water extract. Also, Raman *et al*. (2016) reported 187.23 mg QE/ g of TFC from *M. alba* using 70% ethanol which higher than the current study. Similarly, Han and Zhang reported 2.10 mg/g of TFC which was lower than the current finding 35.67 ±1.54mg QE/g. Mahmood *et al*. (2017) reported TFC value of Mulberry fruits ranges from 58–1021 mg/100 g CE which was lower than the current findings because of ripening stage and drying conditions have a significant impact on the polyphenols. The specific phytocompunds present in wild edible fruits may also absorb at 515nm in a spectrophotometer. Phytocompounds like anthocyanins, carotenoids and chlorophyll degradation products like pheophytin and phytol may increase the absorption that increases the value of total flavonoid content of fruits (Chandra *et al*., 2021). In addition, the TFC of *P. acidus* of current finding was 60.6±0.13mg QE/g, which is in between the values reported by Rahman *et al*. (2011) i.e. 24.18mg QE/g and Moniruzzaman *et al*. (2015) i.e. 168.24 mg QE/g of dried extract. The variance in the TFC value of fruit is due to geo-location, biological parameters, the processing technique used during analysis, and many other aspects (Bhusal *et al*., 2020*)*.

Ascorbic acid (Vitamin C) is a significant phytochemical component in many plant sections, with high antioxidant effects crucial for human health. In the current study, the value of ascorbic content ranged from 4.09±0.34 to 25.11±0.27 mg/g of dry fruit. The highest amount of ascorbic acid was found in *P. acidus, and the* least was found in *P. crenulata*. Sabir *et al*. (2017) reported 217.7±3.1 to 400±2.4 mg/100 g of ascorbic acid from *P. emblica*, which were lower than the current study. A study by Pal *et al*. (2013) reported the vitamin C content of *P. crenulata* and *B. asiatica* was 18.68± 0.891 mg/ g and 31.96± 1.524 mg/g of extract, which was slightly higher than the current study i.e. 4.09±0.34 mg/g and 15.41± 0.1 of dry extract. The vitamin C concentration of all the wild edible fruits in this study varied, which might be attributed to a variety of factors such as plant ages and climate variations, as well as species differences from various geographic areas (Narzary *et al*., 2015).

Tetraterpene pigments known as carotenoids are found in a variety of photosynthetic living things. In the non-photosynthetic organs of plants, they function as color attractants, antioxidants, photoprotectors, and precursors of plant hormones. Since carotenoids cannot be synthesized by animals, they must either be obtained from diet or somewhat altered by metabolic processes (Maoka, 2020). From the current research, only two types of carotenoids (beta-carotene and lycopene) were quantified from wild edible fruits. Both β-carotene and lycopene were calculated using the formula mentioned in Chapter III. Both β-carotene and lycopene were weakly detectable in nearly all fruit samples. β-carotene ranges from 0.02±0 to 0.17±0.01 mg/g of dry extract, whereas lycopene ranges from 0.01±0 to 0.06±0.01 mg/g of dry extract. Bhusal *et al*. (2020) reported that beta-carotene of *Berberi*s species ranged from 0.05 ± 0.01 to 0.06 ± 0.03 mg/g and lycopene content ranged from 0.02 ± 0.01 to 0.04 ± 0.01 mg/g which were quite similar to our findings. In addition, Khoo *et al*. (2008) reported a similar amount of carotenoids from fourteen types of undernutralized fruits from Malasiya. It is well established that cultivar, growth seasons, UV exposure, and maturation phases all affect how much lycopene and carotenoids are present in different fruits (Lee *et al*., 2005; Brandt *et al*., 2006).

The DPPH (1, 1-diphenyl-2-picryl hydrazyl) assay is one of the most reliable techniques for evaluating the potential of antioxidents present in a sample to scavenge radicals through the hydrogen atom transfer mechanism (Huang *et al*., 2005). In comparison to standard ascorbic acid, the DPPH technique analysis of wild edible fruits revealed a significant potential for free radical scavenging. In this current research, *P. emblica* and *B. asiatica* showed the highest radial scavenging potential. The quantity of sample needed to generate 50% of the DPPH free radical scavenging activity is referred to as the total antioxidant activity (IC_{50}), which determines the antioxidant activity of sample. The study revealed that *P.emblica* of methanolic extract had maximum antioxidant potential with an IC_{50} value of 2.35 µg/mL (R^2 = 0.9703), followed by *B. asiatica* with an IC_{50} value of 12.03 µg/mL (R^2 = 0.9828), whereas, *Morus* sp. had the lowest antioxidant potential with an IC_{50} value of 95.08 µg/mL (R^2 = 0.9841). The current study revealed the IC_{50} value of *P. crenulata* was 34.53 µg/mL from methanolic extract, whereas Pandey *et al*. (2023) and Tewari *et al*. (2020) reported 18.75 µg/mL and 115.29 µg/mL, respectively, from ethanolic extract. In previous studies, Yang and Lee (2012) reported an IC_{50} value of 93.8±1.2 µg/mL from *M. alba*, and Bang et *al*. (2007) reported 38.5 µg/mL and 42.2 µg/mL from 60% and 80% ethanol extracts, respectively. Similarly, *P. acidus* having maximum vitamin C among the fruits does not show maximum IC_{50} value among fruits because the RSA% can be influenced by a combination of factors, including the presence of other antioxidants, processing and storage effects, interactions between compounds, the presence of prooxidants, measurement methods, and environmental factors. Environmental factors that affect the amount of secondary metabolites in plants, such as phenol and flavonoids, can be linked to the antioxidant activities of fruits and other plant species. These factors include water, air, soil, elevation, genetic differences between species, extraction methods, and antioxidant measurement (Zargoosh *et al*., 2019).

The antimicrobial activity of all wild edible fruit was determined by the agar-well diffusion technique and examined on ATCC cultures of seven common human pathogens: *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae*, *Samonella typhi*, *Bacillus subtilis*, *Enterococcus faecalis*, and *Candida albicans*. The methanolic extracts of *P. crenulata, P. emblica, B. asiatica, R. paniculatus,* and *M. semiserrata* exhibited significant antibacterial activity against all seven pathogens because of the presence of terpenoids. Terpenoids can exert antimicrobial effects through multiple mechanisms. These mechanisms may include disruption of microbial cell membranes, inhibition of essential enzymes or proteins, interference with microbial biofilm formation, and modulation of microbial gene expression. Terpenoids may also act synergistically with other antimicrobial compounds, such as antibiotics or other plant secondary metabolites (Huang *et al*., 2022). According to Saklani and Chandra (2014), *P. crenulata* exhibited the zone of inhibition against E. *coli* (11mm), *K. pneumonia* (11mm), *S. aureus* (11mm), and *C. albicans* (11 mm), which were similar to our findings, i.e., 13mm, 8mm, 10 mm, and 12 mm, respectively, at 50 mg/mL. In addition, data from Shraddha *et al*. (2018) and Gaur *et al*. (2017) supported current research where *B. asiatica* showed a significant zone of inhibition against *B. subtilis* (8mm) and *S. aureus* (9mm) at 50 mg/mL. Likewise, Varghese *et al*. (2013) also reported that *P. emblica* performed a similar type of zone of inhibition against *S. aureus* and *E. coli.* The ZOI was observed to be higher in gram-positive bacteria than in gram-negative bacteria because of the thicker lipopolysaccharide layer in the outer membrane of gram-negative bacteria, which serves as a defense material against antibacterial substances. The inhibitory property of wild edible fruits concentrates may be due to the presence of phytochemicals, organic acids, and polyphenols, which showed a significantly positive correlation with the value of phytochemicals observed in wild edible fruits (Rios and Recio, 2005).

CHAPTER VI: CONCLUSIONS AND RECOMMENDATION

6.1 Conclusions

From the current study, it can be concluded that *A. macrocarpa* had the highest carbohydrate and protein content among the wild edible fruits examined. Similarly, fat and ash content were found to be highest in *M. semiserrata* fruits. Likewise, TPC, TFC, and vitamin C were found best in *P. emblica* and *P. acidus* fruits. Among all wild edible fruits, *P. emblica* and *B. asiatica* demonstrated good antioxidant activity. In addition, methanolic extracts of *P. crenulata, P. emblica, B. asiatica, R. paniculatus,* and *M. semiserrata* exhibited significant antimicrobial activity against all seven human pathogens, indicating that they have the potential to be an essential source of antibiotic resistance. The findings suggest that freshly consumed wild edible fruit is rich in nutrients and also has natural antioxidant and antimicrobial potential, which can significantly contribute to addressing the nutritional needs and potential use in medicine and value added products.

6.2 Recommendations

- This investigation has shown that, in comparison to the water extracts, the methanolic extract contains a higher concentration of phytochemicals. Consequently, in order to achieve better results, methanol is advised when extracting plant components.
- Due to the significant medicinal value attributed to the phytocompounds present in these plant species, more research needs to be done to separate, identify, describe, and clarify the structures of these bioactive principles and maximize their potential for use in industry and medicine.
- Even though the majority of wild edible fruits and their extracts have been used for millennia, there is still a lack of variety in the nutritional and therapeutic applications of these fruits. That being said, it is crucial to appropriately record them and carry out valuable research.
- It is recommended that a range of standardized methods and tools, including GC-MS and HPLC, be employed to thoroughly examine and identify the valuable chemical compositions found in WEFs. This will enable their classification based on their significance to the food and pharmaceutical industries.

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APPENDICES

APPENDIX A

A. List of materials, media, chemicals and reagents:

a) Equipments:

1. Autoclave	2. Incubator
3. Spectrophotometer	4. Hot air oven
5. Refrigerator	6. Muffle furnace
7. Weighing machine	8. Vortex machine
9. Water Bath	10. Elisa plate
11. Biological Safety Cabinet	12. Grinder
13. Shaking Incubator	14. Soxhlet apparatus

b) Glass Wares:

1. Beakers	2. Petri dishes
3. Conical Flask	4. Micropipette
5. Volumetric flask	6. Test tubes
7. Glass vails	8. Measuring Cylinders
9. Test tubes	

c) Microbiological Media:

1. Nutrient agar	2. Muller Hinton agar
3. Nutrient broth II	4. McFarland

d) Chemicals and Reagents:

1. Methanol	2. Ascorbic acid
3. 2,2-Diphenyl-1-Picryl hydrazyl (DPPH)	4. Hydrochloric acid (HCl)
5. Sulphuric acid (H2SO4)	6. Sodium Hydroxide (NaOH)

7. Bovine Serum Albumin (BSA)
8. Bradford's Reagent
9. Anthrone Reagent
10. Aluminum Chlorate (AlCl3)
11. Sodium Carbonate(Na2CO3)
12. Gallic acid
13. Quercetin
14. Folin- Ciocalteau
15. Perchloric acid
16. Phosphoric acid
17. Ethanol
18. Glucose

e. Miscellaneous

1. Innoculating loop and wire
2. Labeling tape
3. Dropper
4. Cotton
5. Cotton swabs
6. Forceps
7. Parafilm Tape
8. Scale
9. Permanent Marker
10. Aluminum foil
11. Butter paper

APPENDIX B

B. Composition of cultural media:

The culture media used were from Hi-Media Laboratories Pvt. Limited, Bombay, India (All composition are given in gram per liter and 25° C (Temperature)

1. Nutrient Agar (NA):

S.No.	Ingredients	gram/litre
1.	Peptic digest of animal tissue	5
2.	Beef extract	1.5
3.	Yeast extract	1.5
4.	Sodium chloride	5
5.	Agar	15

Final pH (25°C) 7.4±0.2

Preparation: As directed by manufacturer, 38 g of the medium was dissolved in 1000 mL of dissolve water and then boiled to dissolve completely. The medium was autoclaved at 121° C (15 lbs. pressure) for 15 minutes. The sterilized medium was then poured in sterilized Petri- dishes and was allowed to cool.

2. Nutrient broth (NB):

S.No.	Ingredients	gram/litre
1.	Peptic digest of animal tissue	5
2.	Beef extract	1.5
3.	Yeast extract	1.5
4.	Sodium chloride	5

Final pH (25°C) 7.4±0.2

Preparation: 13 g of the medium was suspended in 1000 mL of distilled water. Then, 5 mL of medium was dispensed in each test tube and sterilized at 121° C for 15 minutes at 15 lbs. pressure. The sterilized medium was then cooled to room temperature.

3. Muller- Hinton Agar (MHA):

S.No.	Ingredients	gram/litre
1.	Beef infusion form	300
2.	Casein acid hydrolysate	17.5
3.	Starch	1.5
4.	Agar	17

Final pH (25° C) 7.3±0.1

Preparation: As directed by manufacturer, 38 g of the medium was dissolve in 1000 mL of distilled water and then boiled to dissolved completely. The medium was autoclave at 121° C (15 lbs. pressure) for 15 minutes. The sterilized medium was the poured in sterilized petri- dishes and was allowed to cool.

4. Preparation of 0.5 McFarland standards

99.4 mL of 1% v/w sulfuric acid solution was mixed with 0.6 mL of 1% w/v barium chloride solution. Next, the mixture was thoroughly shaken. This stock solution was kept in a dark, well-sealed container. To compare the turbidity of the inoculums, a small portion of the solution was collected and placed in clean test tubes.

APPENDIX C

DIFFERENT REAGENTS: COMPOSITION AND PREPARATION

C. Preparation of reagents:

a) Preparation of different concentration of plant extract for antimicrobial activity

1 g of plant extract was dissolved in 1 mL of dimethyl sulfoxide (DMSO) to prepare the concentration of 1000 mg/mL. Then different concentration (50 mg/mL, 100 mg/mL, 150 mg/mL and 200 mg/mL) were made using formula: S1V1 = S2V2

Example: for 200 mg/mL 200 µL of stock solution from 1000 mg/mL was transferred to microfuge tube and 800 µL of DMSO was added to it.

b) Preparation of 0.3mM DPPH solution

5.9mg of DPPH was dissolved in 50 mL of methanol. It was protected from light by covering the volumetric flask with aluminum foil.

c) Preparation of L-Ascorbic acid

10 mg L-Ascorbic acid was dissolved in 10 mL methanol to prepare stock solution of 1000 µg/mL. Test solution of 5, 10, 15, 20, 25 µg/mL of ascorbic acid was prepared by diluting from stock solution of concentration 1000 µg/mL.

d) Preparation of test sample of antioxidant

10mg of sample dissolve in 10mL of methanol solution prepare stock solution of 1000 µg/mL (mL equivalent to mg). Test solution 5, 10, 15, 20, 25 µg/mL of plant extract was prepared by diluting from stock solution of 1 mg/mL using formula S1V1 = S2V2

e) Preparation of Bradford reagent for protein analysis:

10 mL of 85% (w/v) phosphoric acid (H_3PO_4) was added after 5 mg of Coomassie Brilliant Blue G-250 had been dissolved in 5 mL of methanol. Next, the dye was allowed to dissolve completely in the 100 mL volumetric flask before 85 mL of water was gradually added. Right before using, filter with Whatman No. 1 paper to get rid of the precipitates, then store in a dark bottle at 4°C. The volumetric flask was covered with aluminum foil to keep light from reaching it.

f) Preparation of anthrone reagent for carbohydrate analysis:

25mg of solid crystal anthrone was dissolved in 12.5mL of conc.H_2SO_4 (i.e, 2:1 ratio) and shake vigorously and stored in cap glass bottle.

g) Preparation of 10% sodium carbonate (Na_2CO_3) solution:

10g of sodium carbonate powder was taken in clean volumetric flask having a capacity of 100mL and pour the distilled water within the flask until the lower meniscus of water touch the label line. Vortex up to one minute and store in capped glass bottle.

h) Preparation of 2% Aluminum chloride ($AlCl_3$) solution

2 g of $AlCl_3$ was place in 100 mL volumetric flask and dissolve it by adding require amount of water then vortex up to one minute and store in capped glass bottle.

i) Preparation of gallic acid and Quercetin

10 mg of each gallic acid and quercetin was dissolved with 10mL methanol in each glass vials. Vortex upto 1 minute and solution was filtered through Whatman No. 1 paper.

j) Preparation of D-Glucose and Bovine Serum Albumin

10 mg of each D-Glucose and BSA was dissolved with 10 mL water in each glass vials. Vortex up to 1 minute and used for the standard curve.

k) Preparation of 52% perchloric acid

433.3mL of 60% purity perchloric acid was measured with measuring cylinder and 66.6 mL of distilled water was measured in volumetric flask of 500mL capacity.

l) Preparation of 1% metaphosphoric acid

1 g of metaposphoric acid was dissolved in 100mL of distilled water.

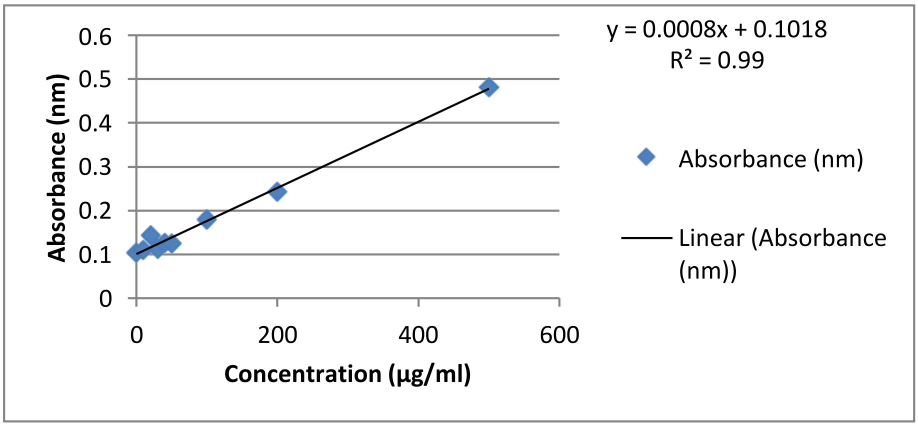
m) Preparation of 0.01% of 2, 6-dichlorophenolindophenol (DCPIP)

0.005 g of DCPIP was dissolved in 50 mL of distilled water. It was protected from light by covering the volumetric flask with aluminum foil.

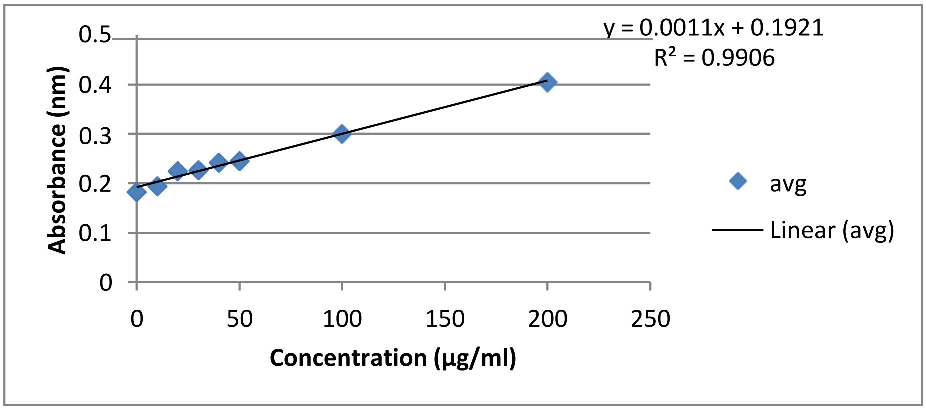
APPENDIX D

D. Calibration curve of standards

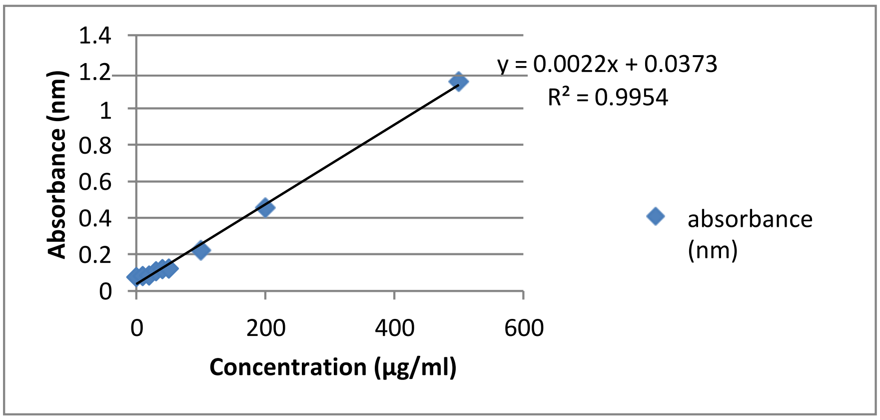
a. The absorbance curve for standard glucose for carbohydrates test:



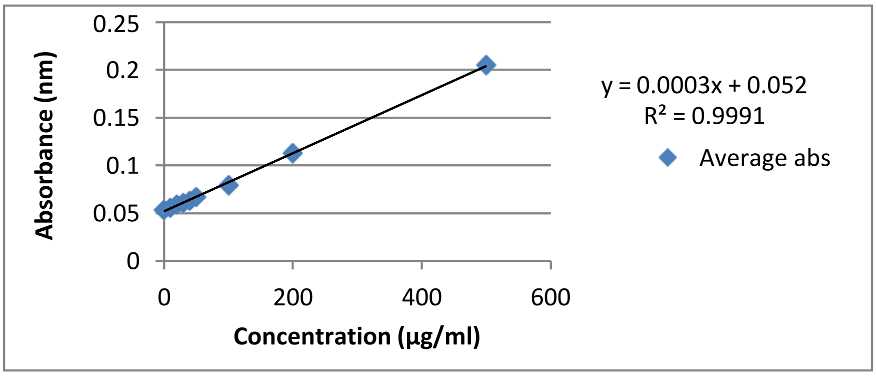
b. The absorbance curve for standard Bovine Serum Albumin (BSA) for protein test:



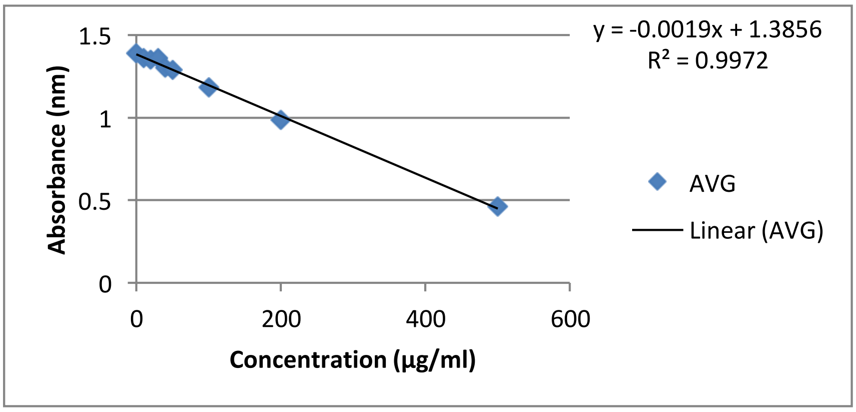
c. The absorbance curve for standard gallic acid for total phenolic content:



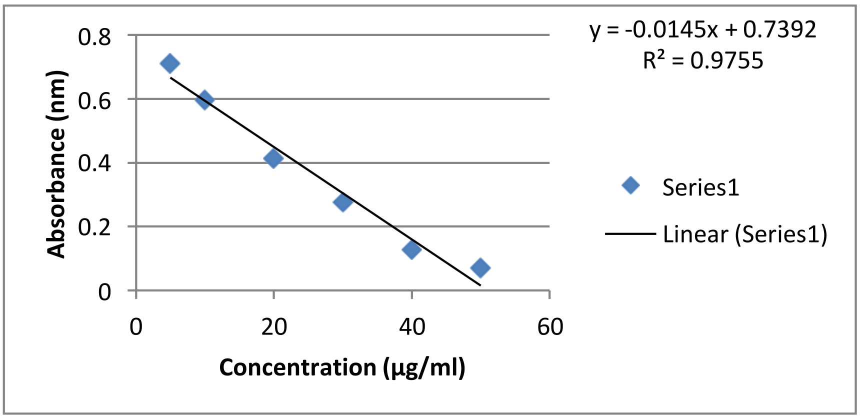
d. The absorbance curve for standard quercetin for flavonoid content:



e. The absorbance curve of Ascorbic acid for Vitamin C content :

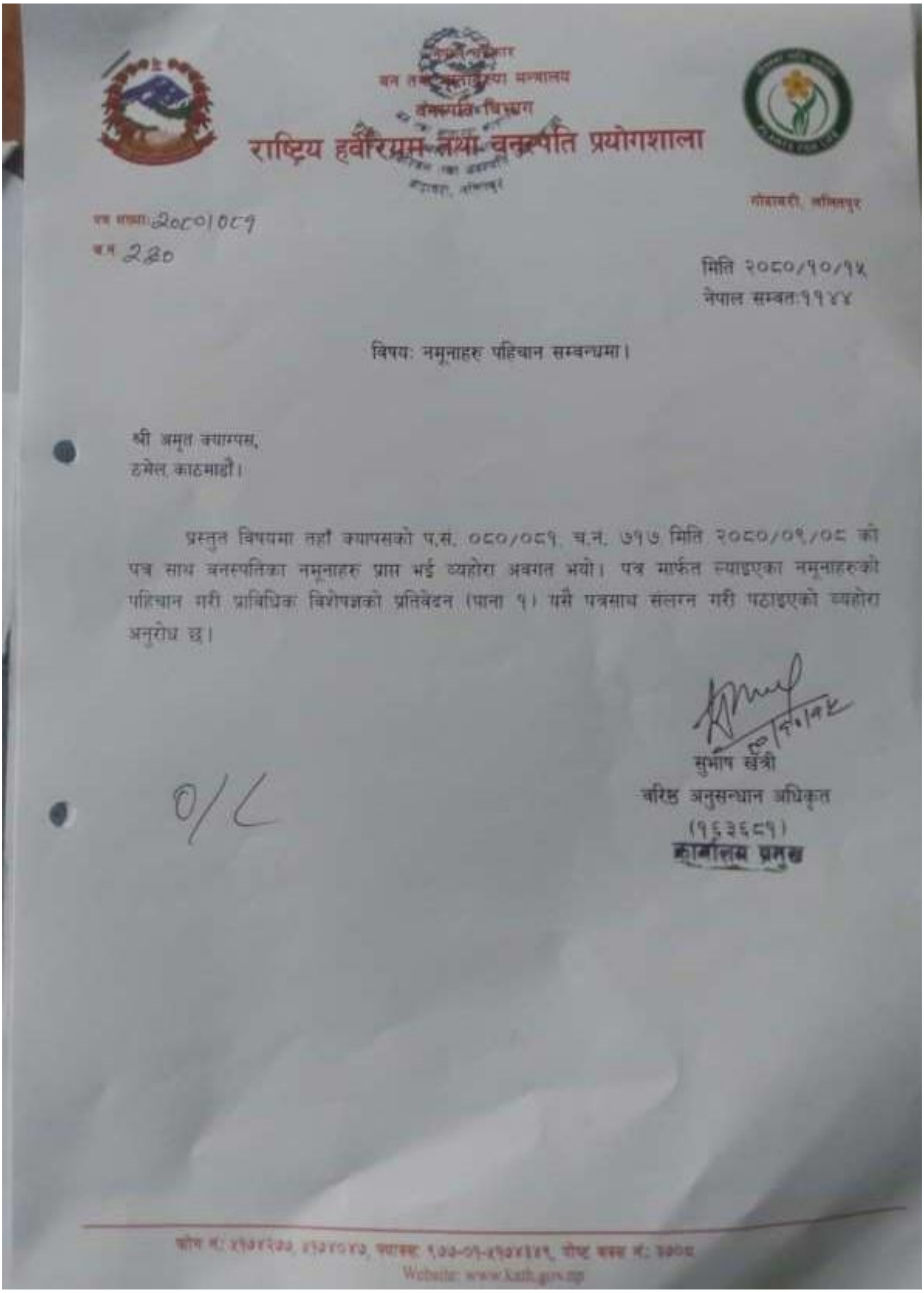


f. The absorbance curve of ascorbic acid for antioxident activity:



APPENDIX E

E. Herbarium conformation letter from National Herbarium and Plants Laboratory



राष्ट्रिय हर्बेरियम तथा वनस्पति प्रयोगशाला

गोदावरी, ललितपुर

पत्र संख्या: २०८०/०८१
च.नं. २३०

मिति २०८०/१०/१५
नेपाल सम्वत ११४४

विषय: नमूनाहरु पहिचान सम्बन्धमा।

श्री अमृत क्याम्पस,
ठमेल, काठमाडौं।

प्रस्तुत विषयमा तहाँ क्यापसको प.सं. ०८०/०८१, च.नं. ७१७ मिति २०८०/०९/०८ को पत्र साथ वनस्पतिका नमूनाहरु प्राप्त भई व्यहोरा अवगत भयो। पत्र मार्फत ल्याइएका नमूनाहरुको पहिचान गरी प्राविधिक विशेषज्ञको प्रतिवेदन (पाना १) यसै पत्रसाथ संलग्न गरी पठाइएको व्यहोरा अनुरोध छ।

८०/१०/१५

सुभाष खत्री
वरिष्ठ अनुसन्धान अधिकृत
(१६३६८१)
कार्यालय प्रमुख

O/C

प्राविधिक विशेषज्ञको प्रतिवेदन

१. नमूना परिक्षण गर्न पठाउने व्यक्ति/निकाय:- श्री अमृत क्याम्पस, लैनचौर काठमाडौं।

१(क) विद्यार्थीहरुको नाम:- श्री एलिना राई

२. प्राप्त नमूनाको विवरण:- बनस्पतिका नमूनाहरु ११ थान।

३. यस कार्यालयमा प्राप्त मिति:- २०८०/०९/२०

४. परिक्षणका आधारहरु:- (क) हर्बेरियममा भएका नमूनाहरु संगको तुलनात्मक अध्ययन ।
(ख) सन्दर्भ सामग्रीहरुको अध्ययन ।

५. पहिचान प्रतिवेदन:- प्राप्त नमूनाको Morphological अध्ययन र यस राष्ट्रिय हर्बेरियम तथा बनस्पति प्रयोगशालाको हर्बेरियममा राखिएका नमूनाहरु संगको तुलनात्मक अध्ययन गर्दा उक्त नमूनाहरु निम्नानुसार भएको पहिचान हुन गएको।

S.N	Scientific Name	Ref. No.	Family	Remarks
1	*Morus alba* L.	01	Moraceae	
2	*Berberis asiatica* Roxb. ex DC.	02	Berberidaceae	
3	*Averrhoa carambola* L.	03	Oxalidaceae	
4	*Ardisia macrocarpa* Wall.	04	Primulaceae	
5	*Phyllanthus acidus* (L.) Skeels	05	Phyllanthaceae	
6	*Myrsine semiserrata* Wall.	06	Primulaceae	
7	*Morus nigra* L.	07	Moraceae	
8	*Morus* sp.	08	Moraceae	
9	*Rubus paniculatus* Sm.	09	Rosaceae	
10	*Pyracantha crenulata* (D.Don) M.Roem	10	Rosaceae	
11	*Smilax aspera* L.	11	Smilacaceae	

६. परिक्षण गर्ने अधिकारी:-

तिल कुमारी थापा
अनुसन्धान अधिकृत
(२३३३४९)

PHOTOPLATES

1. *Pyracantha crenulata*

2. *Phyllanthus acidus*

3. *Phyllanthus emblica*

4. *Rubus paniculatus*

5. *Morus nigra*

6. *Morus alba*

7. *Morus* sp.

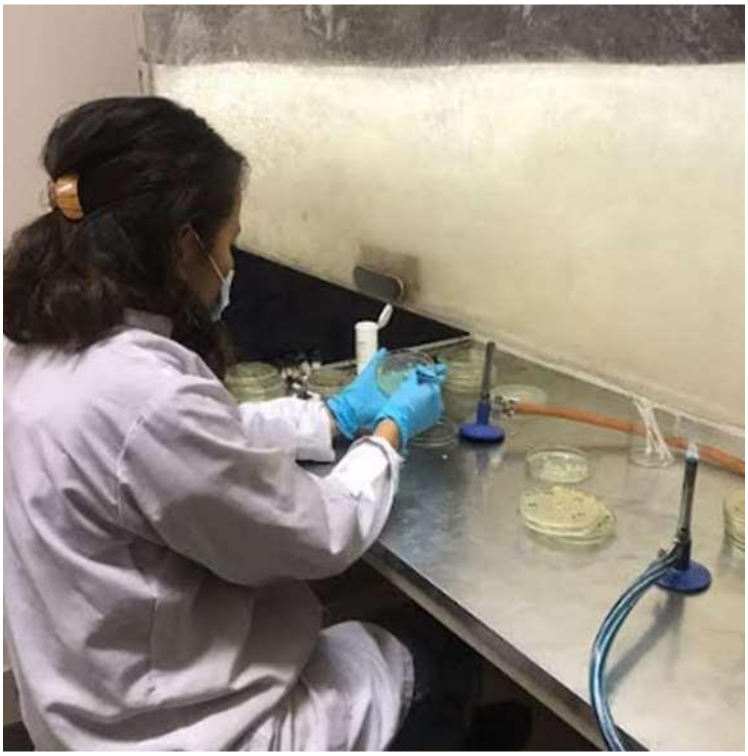
8. *Smilax aspera*

9. *Ardisia macrocarpa*

10. *Averrhoa carambola*

11. *Berberis asiatica*

12. *Myrsine semiserrata*

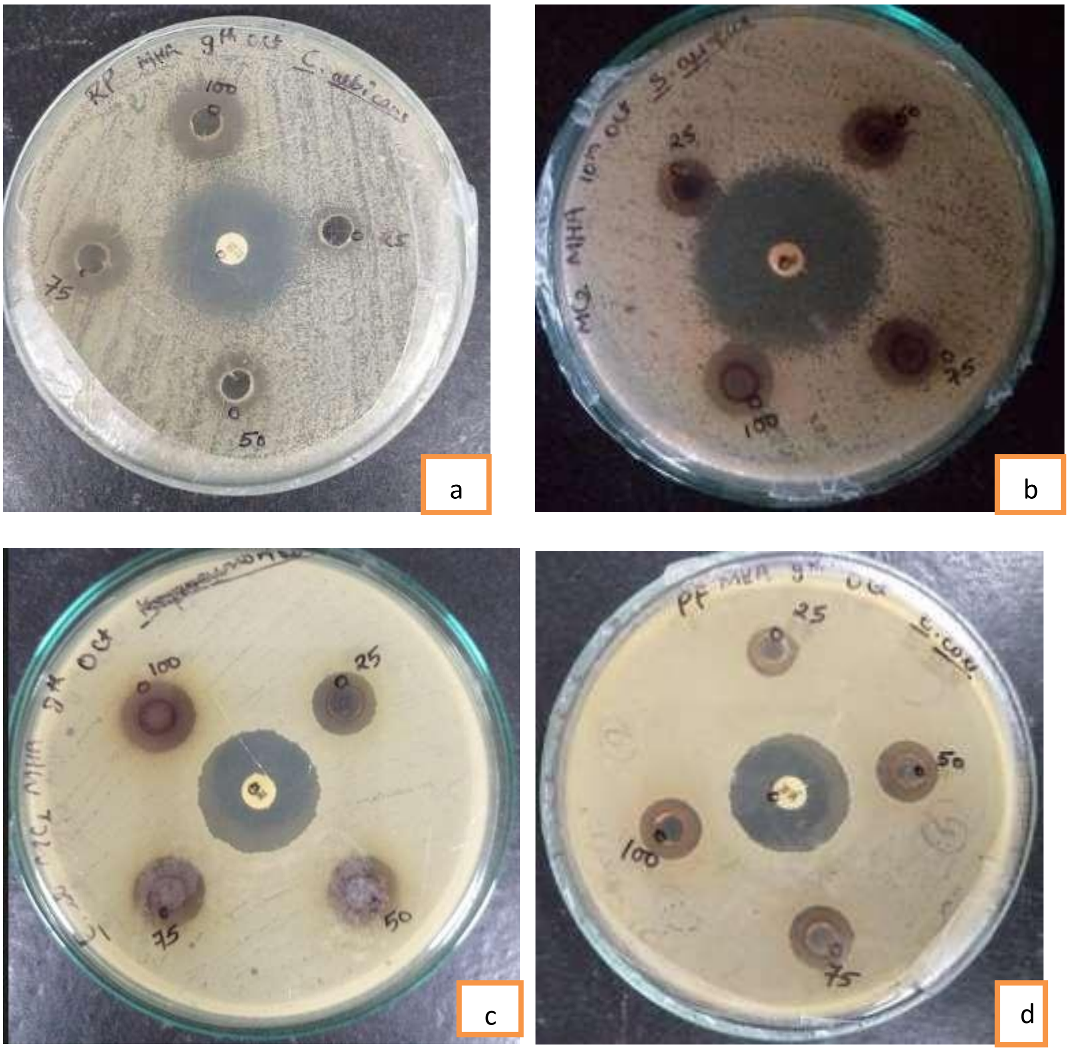


Performing antibacterial test in Biosafety cabinet

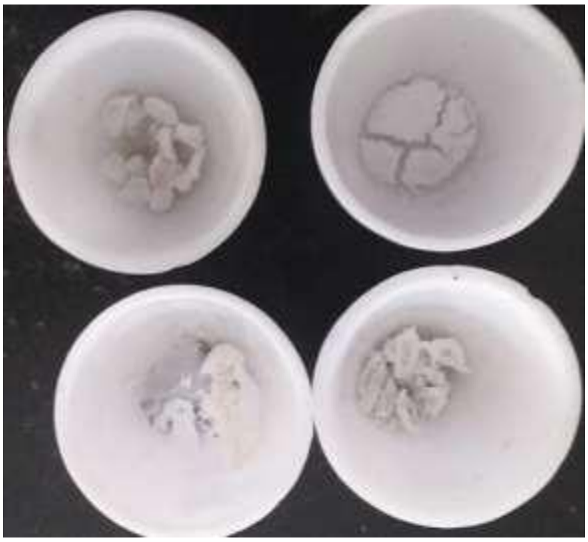
Water extract of fruit samples after filtration

Antioxidant test performed in ELISA plate

Methanolic extract of fruit samples after filtration



Antimicrobial activity of a) *R. paniculatus* in *C. albicans* b) *M. semiserrata* (B) in *S. aureus* c) *M. semiserrata* (A) in *E. coli* and d) *P. crenulata* in *E.coli*



Ash content of fruit samples after taking out from muffle furnace

EVALUATION OF NUTRIENT VARIABILITY, ANTIOXIDANT POTENTIAL AND ANTIMICROBIAL ACTIVITY OF SELECTED WILD EDIBLE FRUITS

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