EXTRACTION OF ANTHOCYANIN PIGMENT FROM SELECTED PLANT SOURCES AND ITS FOOD APPLICATION

Dissertation submitted to

ST. TERESA'S COLLEGE, ERNAKULAM

(Autonomous)

Affiliated to

MAHATHMA GANDHI UNIVERSITY, KOTTAYAM

In partial fulfillment of the requirement for the award of the

DEGREE OF MASTER OF SCIENCE IN

FOOD SCIENCE AND NUTRITION

By

DENISHYA MARY JACOB

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Department of Home Science and Centre for Research

St. Teresa's college (Autonomous)

Ernakulam

June 2023

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CERTIFICATE

I hereby certify that the dissertation entitled '**Extraction of Anthocyanin Pigment from Selected Plant Sources and its Food Application'** prepared and submitted by Ms. Denishya Mary Jacob is an original research work carried out under my guidance and supervision.

Signature of the Head of the Department

Dr. Shilpa Jose

Assistant Professor

Department of Home Science

St. Teresa's College

Ernakulam

DECLARATION

I hereby declare that this research work entitled '**Extraction of Anthocyanin Pigment from Selected Plant Sources and its Food Application'** is an original research work carried out by me under the supervision and guidance of Dr. Shilpa Jose, Assistant Professor, Department of HomeScience, St. Teresa's College Ernakulam.

Place: **Denishya Mary Jacob**

Date:

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INTRODUCTION

CHAPTER I

INTRODUCTION

Colour has been identified as one of the most vital properties that determine the acceptability of beverages and foods. Since the colour in food is mostly due to the appearance of pigments, synthetic colourants are often employed to provide an acceptable colour in the final food product. Synthetic colourants are widely employed in many processed foods as they are highly durable over time. Additionally, it was discovered that synthetic pigments could potentially be poisonous that can result in a negative impact on human health. Therefore, there is a rising demand for the use of plant-derived extracts as a result of concerns about the safety of synthetic colourants, improved consumer knowledge, and the commercial availability of natural colourants. Natural pigments, in contrast, have been shown to perform beneficial biological activities like antioxidant and anti-inflammatory qualities. They are also extremely safe for human health. The most prominent naturally-occurring plant-derived pigments are anthocyanins, betalains, carotenoids, and chlorophyll. (Nazila Ghareaghajlou, 2021)

Among many types of natural pigment of plant origin, anthocyanin represents the largest group of water-soluble pigments. Anthocyanins are polyphenol substances that provide flowers, vegetables, and fruits with their distinct hues of pink, red, purple, and blue. Because anthocyanins are abundant in nature and have high health-promoting advantages, such as anti-inflammatory; and anti-diabetic properties, high compatibility to biological systems, and nontoxicity, they can be considered the best natural colourants to be used in the food industry. (Saqib Farooq, 2020) A number of scientific researches have been conducted on phenolic substitutes for artificial food additives and their beneficial effects on human health resulting from their antioxidant qualities.

Anthocyanins are water-soluble phenolic chemicals found in a wide variety of vegetable products that are responsible for a wide spectrum of colours ranging from colourless to purple. The Anthocyanins are thought to be the biggest, most interesting, and most intriguing group of plant-based pigments used by humans historically as colouring agents for foods, drinks, and clothing, as well as for baits, armour, phytopharmaceuticals, colours for drawings and cave art, and for celebrations. The Greek words for anthocyanin are anthos, which means flower, and kyaneos, which means dark blue colour. They are a group of very efficient bioactive compounds that are widely distributed in plant food. Anthocyanins occur in all plant tissues, including

leaves, stems, roots, flowers, and fruits. Despite having few chromophore groups, over 540 naturally occurring ring anthocyanidin pigments have been found. Their main role in plants is to attract living beings, particularly insects and birds, for pollination and seed dispersal functions. The nature and concentration of anthocyanins in fruits, flowers, and vegetables determine their colour variances. (María de Lourdes Vargas y Vargas, 2013)

Anthocyanins are oxygenated heterocycles with two aromatic rings joined by three carbons (i.e., a chromane ring with a second aromatic ring in position 2). The processes of hydroxylation, methylation, glycosylation, and acylation are used to modify anthocyanins. As a result, anthocyanins' colours and stability are made more adaptable. The anthocyanin's hue deepens in blue as the B-ring's number of hydroxyl groups rises. On the other hand, methylation causes anthocyanins' colour to change towards red. The anthocyanins become less susceptible to oxidation and are stabilised due to methylation of the B-ring, affecting the stability of anthocyanin. Anthocyanin molecules can undergo metabolism and degradation in either the A or B ring (Tian Jiang, 2018). In a study on the metabolism of anthocyanins, it was found that the methoxy group boosted the anthocyanins' stability during simulated gastric digesting processes by replacing the hydroxyl groups of the B-ring. (Luis E. Rodriguez-Saona, 2001)

Anthocyanins are an important part of the food for a healthy lifestyle, and sometimes it is usual to consume fruits to improve health and general well-being. However, they do not meet the requirements of critical nutrients that humans need. A low intake of fruits and vegetables has been linked to an estimated 9%, 11%, and 14% of cases, respectively, where 1.7 million fatalities were brought on by heart failure, ischemic heart disease, and GIT-related cancers (WHO, 2004). In this regard, it has been determined that the daily intake of flavonoids and anthocyanins is between 200 and 250 mg (Taylor C Wallace, 2015), whereas the WHO-FAO has set the intake of anthocyanins derived from grape skin at 2.5 mg/kg. The US-NHANES (United States National Health and Nutrition Examination Survey) supports the US-FDA Nutrient Database's recommendation that anthocyanins intake in the country be limited to 12.5mg per day per individual. Interestingly, no human toxicity of anthocyanin has been reported till now, especially from food intake, the quantity of which is very infinitesimal. Studies have found that nutrient utilization of anthocyanins supplementation in the diets of 7–10 years old children improved their cognitive functions (Adrian R Whyte, 2016), which have also been observed in adults (Lamport Daniel J 2, 2016) , and the elderly (Katherine Kent, 2015)

Six anthocyanidins that are frequently found in nature and have dietary significance are cyanidin, delphinidin, petunidin, peonidin, pelargonidin, and malvidin. The secondary structures of flavylium ion, quinoidal base, carbinol base, and chalcone pseudo base coexist with anthocyanins in solution. Through a variety of stabilizing mechanisms, anthocyanins' self-association, intermolecular, and intramolecular co-pigmentation result in the formation of tertiary structures. Plant taxonomic classification has historically employed anthocyanin composition as a technique in botanical studies. Additionally, anthocyanin profiles of fruits and vegetables are marks the quality of the products and enable the detection of adulteration of anthocyanin-based products. (Jiaqi Tan, 2022)

Anthocyanins are common components of the human diet since they are found in a variety of meals, fruits, and vegetables. Additionally, anthocyanins have antioxidant properties, which mostly depend on their chemical composition. Numerous epidemiological studies have demonstrated the advantages of a diet high in fruits and vegetables for maintaining human health as well as for the prevention of numerous conditions linked to oxidative stress, such as cancer and cardiovascular conditions. Anthocyanins function as antioxidants as a result in the foods that contain them as well as in the body after ingesting these foods. Due to their colouring qualities, anthocyanin-rich extracts are becoming more and more popular in the food industry as natural alternatives to artificial FD&C dyes and lakes. (Julia Martín Bueno, 2012)

The antibacterial properties of anthocyanins have been demonstrated in numerous research studies. Anthocyanin's antibacterial properties are thought to result in the degradation of cell walls, cell membranes, and intercellular matrix. Anthocyanins are also found to exhibit immunostimulatory, and anticancer activities along with being a potent antioxidant. The abilities of anthocyanins to induce apoptosis and suppress angiogenesis were explained as the reasons for the anticancer activities of anthocyanins by the American Association for Cancer Research (Longo, et al., 2008). Anthocyanins in their acylated forms are also efficient to reduce postprandial glucose levels by inhibiting maltase activity, proving anthocyanin can also be used in the management of Diabetes Mellitus.

Anthocyanins are also found to be excellent antioxidants in their natural form, which is controlled by their chemical structures. Anthocyanins were able to act as reducing agents in the electron-transfer reaction pathway with the ability to donate electrons to the free radicals with unpaired electrons. Anthocyanins are some of the strongest antioxidants due to their free radical scavenging abilities. The anti-inflammatory potential of anthocyanins is also explained

by the pathogenesis of obesity (Yoon-Mi Lee, 2017). Anthocyanins have been shown to have anti-inflammatory characteristics and the potential to be employed as innovative therapeutic agents in the treatment of Ulcerative Colitis, which is a major form of inflammatory bowel disease (IBD). (Shiyu Li, 2019)

The Market Data Forecast website states that the market for products containing anthocyanins is expanding at a rate of 4.6% per year and that it will reach 388 million USD in value by 2026 (Market Data Forecast, 2023). Additionally, the businesses that produce beverages, food supplements, and personal care items can benefit from anthocyanins-based applications. As a result, plants high in anthocyanins are gaining interest from the food, immunity-boosting, and healthcare industries. They have also been promoted as food and drug additives with a number of other medications, herbal extracts, and the anthocyanins themselves in specific dosage forms, such as liquids, tablets, and capsules and are sold for the purpose of weight loss, as antioxidant, immune-stimulant and general health well-being.

In the food, beverage, cosmetics, and nutraceutical industries, anthocyanins are quickly becoming one of the most promising components. A shift in anthocyanin research has been brought about by consumer desires for natural food colourants and anthocyanin-based nutraceuticals (Massimo Iorizzo 1 2, 2020). Anthocyanins have been used to create pink, red, purple, and blue colours in a number of applications. Natural components are preferred by consumers since they are thought to have fewer side effects than manufactured or artificial ingredients. As more people are concerned with global sustainability issues at the present day, consumers are also willing to sacrifice on price. Industries are currently considering releasing the best versions of these items from research initiatives into the market and have successfully addressed several storage and stability-related difficulties. The cost of production and a lack of market are two issues currently facing this industry. Consumers also seek convincing data to alter their decisions and thus move from synthetic to natural ingredients.

Due to this lack of a cost-effective sustainable production system for natural food colourants, alternate and cheap sources are a good choice and warranted. Phytoconstituents derived from plants have benefits for health and well-being and offer exciting commercial opportunities. Consumers prefer fruits for their beneficial nutrients and expect value addition associated with their potential bioactivity (Ute Nöthlings, 2008). There is a great impetus to identify new biomolecules with promising activity from fruits, because, as natural ingredients, they have a proven benefit for human health and turn out to be new opportunities for the food

processing industry. Recent studies have shown fruits from less-known and unexplored plants as excellent sources of nutrients and biologically active compounds for food and non-food applications (Mohammad Imtiyaj Khan, 2013)

Therefore, in this study entitled “Extraction of anthocyanin from selected plant sources and its food application” anthocyanin pigment from 5 different plant sources is obtained, namely Basella alba fruit, Carissa carandas, Cockscomb, Dragon fruit peel and Banana bract. The objectives of this specific study are

- To extract anthocyanin pigment from selected plant sources as samples for the study.
- To determine and compare the anthocyanin content from selected plant sources.
- To assess the biological properties and stability of anthocyanin pigment from plant sources with its highest content.
- To study the application of anthocyanin in a food product.
- To evaluate the sensory attributes of the newly developed food product.

REVIEW
OF LITERATURE

CHAPTER II

REVIEW OF LITERATURE

The review of literature pertaining to the present study entitled **"Extraction of anthocyanin from selected plant sources and its food application"** is discussed under the following heads.

2.1 Definition and functions of anthocyanin in plants

2.2 Anthocyanins: from plant sources to human health

2.3 Industrial Application of Anthocyanin pigment

2.4 An Overview of Basella alba fruit

2.1 DEFINITION AND FUNCTIONS OF ANTHOCYANIN IN PLANTS

Anthocyanins (Greek: antos, flower and kyaneos, blue) are water-soluble, coloured chemicals of the flavonoid class that are widely distributed in the fruits, leaves, roots, and other tissues of plants (Ashya Shaik, 2018). The processes of hydroxylation, methylation, glycosylation, and acylation are the most used techniques in the modification of anthocyanins. As a result, the colours and stability of anthocyanin are made more versatile. For instance, aromatic acylation and hydroxylation cause anthocyanin pigment to move towards a blue hue, whereas methylation causes the pigment, which is frequently seen on the surfaces of leaves and flowers, to shift towards a red hue. Blueness is increased with an increased number of hydroxyl groups, while redness increases with enhanced methylation in the B-ring (Tanaka Y, 2008) (Alappat, 2020).

Chemically, anthocyanins are mono- or diglycosides of anthocyanidins, which are salts of 2-phenylbenzopyrylium (flavylium) of polyhydroxyl and polymethoxyl derivatives (Julia Martín, 2017). Naturally occurring types of anthocyanidins are produced via substitution at various positions in the flavylium cations. Cyanidin (30%), delphinidin (22%), pelargonidin (18%), peonidin (7.5%), malvidin (7.5%), and petunidin (5%), are the six most abundant anthocyanidins among all the identified anthocyanin pigments in plants. Anthocyanins' chemical structure makes them suitable for use as antioxidants since they can donate hydrogen or electrons to the free radicals or capture and delocalize them in their

aromatic structure (FLAVANOIDS - CHEMISTRY, BIOCHEMISTRY AND APPLICATION, 2006).

Anthocyanins are susceptible to temperature, pH, and light [23,24]. Anthocyanins change their colour to yellow or colourless degradation products under these conditions. Several mechanisms have been proposed to ensure the stability of anthocyanins. The stability and solubility of anthocyanins are also improved by acylation. Acylated anthocyanins can be viewed as covalently bound co-pigmented anthocyanins where copigmentation is a phenomenon in which pigments associate with other compounds (copigments) that results in a colour enhancement or shift, by blocking chromophores from hydration (Raymond Brouillard, 2009).

In plants, Anthocyanidin synthesis (like other flavonoids) occurs on the cytoplasmic leaflet of the endoplasmic reticulum and then they are accumulated in the large central vacuoles (Grotewold E, 2008) of almost every cell type in the epidermal, ground and vascular tissues of all vegetative organs, thereby reaching the peak level at the time of their ripening age (Zhenyu Huang, 2019). They occur in roots, both subterranean and aerial, and in hypocotyls, coleoptiles, stems, tubers, rhizome, stolon, bulbs, corms, phylloclade, axillary buds, and leaves. Each plant and its parts have considerable variations in their anthocyanins content as well as in its chemical structures as these characteristics highly depend on the temperature, light and agronomic factors along with the type of plant species and variety, plant growth stages, and storage conditions of plant-derived products (Li Yang, 2018).

The presence of anthocyanins in plants benefits the plants by acting as a protective barrier against high light intensity and UV-B light. As an antioxidant component of plants, they also perform the primary defence mechanism in plants against a variety of abiotic challenges, including drought and high salt conditions, as well as heat and light impacts. Anthocyanins are also involved in the functioning and regulation of senescence, leaf temperature, osmotic balance, monosaccharide transport, and camouflage. Therefore, they serve an important role in shielding plants' photosynthetic system from excessive light radiation flux (Gould & Lister, 200) and avoiding DNA damage. The anthocyanins also regulate the plant haemostasis and provide protection from drought, heat, cold, and water stress. In addition, it also plays a function in shielding plants from various biotic stressors, such as microbial and insect attacks along with attracting insects for better pollination.

Therefore, anthocyanins have been regarded as both a symptom and a coping mechanism of plants, when under stressful environments, though some authors also report their involvement in the photosynthetic process (Kevin Gould, 2009).

2.2 ANTHOCYANINS: FROM PLANT SOURCES TO HUMAN HEALTH

Anthocyanins are widely distributed in nature and have gathered the attention of the scientific community mostly due to their vast range of possible applications. They have been the focus of research in a variety of sectors, including food development, where their natural colouring, antioxidant capacity, and biological potential present exciting opportunities for the creation of novel food additives and functional foods. Since anthocyanins are naturally occurring and have a wide range of applications, extracting them from various plant sources, like fruits and flowers, is the most common method of obtaining anthocyanin (S. Oanceaa, 2012).

Isolation of these anthocyanins from plant cells for human use is an important task closely related to the need for the preservation of their bioactivity. Sample size reduction, proper extraction, physical-chemical characterisation, and in vitro studies of a particular biological activity are all steps in the isolation process. For optimization of the composition of mixed extracts to be used in the pharmaceutical, food, or cosmetic industries, it is crucial to choose the right techniques for each phase (Tony K McGhie, 2007).

Anthocyanins were extracted using both conventional and modern (non-traditional) methods, which resulted in either an enriched crude pigment extract derived through a solid-liquid partition process or a more refined extract. Modern extraction techniques, such as pressurised liquid extraction (PLE) and supercritical fluid extraction (SPE), were also applied for anthocyanins (Zhi Yong Ju, 2007), but with only modest success because anthocyanins are heat-sensitive and SFE techniques are particularly suited for non-polar solvents. Conventional extraction of anthocyanins is typically carried out in acetone or acidified methanolic solutions (S. Oanceaa, 2012).

Anthocyanins are likely the most significant subgroup of flavonoids in terms of widespread consumption. According to a recent USDA analysis that evaluated more than 100 common foods, the daily consumption of anthocyanins had previously been estimated to be 180–215 mg per person, but the current estimate is 12.5 mg per person per day in the United States (XIANLI WU, 2006). However, when compared to other phytochemicals with established or hypothesised health advantages, this is a significant quantity. When

blackberry juice was used to treat eye and mouth infections in the 16th century, anthocyanins' positive benefits on human health were at least understood at that time (Dai J, 2007). A wide range of biological activities are displayed by anthocyanins, including their potent antioxidant (Kähkönen MP, 2003), anti-inflammatory, anti-proliferative (Bishayee A, 2010), and anti-carcinogenic (CC, 2007) properties are the fundamental cause of significantly increased interest in the research study of anthocyanin over the past ten years.

The anthocyanin class of flavonoid compounds has a great capacity to scavenge the physiologically generated free radicals at very low concentrations due to the phenolic structural base of these compounds. Due to the anthocyanins' ability to prevent, mitigate, and clean up oxidative stress, they have been shown to have biological activities against a variety of illnesses and physiological malfunctions. These include, in addition to cancer, cardiovascular, neurological, diabetic, eye functions vision (Muth, Laurent, & Jasper, 2000), obesity, inflammation, analgesic and dysentery. In addition to reducing inflammation and altering immune system responses, anthocyanins are also thought to have antioxidant potential. In addition to reducing inflammation and altering immune system responses, anthocyanins are also thought to have antioxidant potential by counteracting the enzymes that attack connective tissues and protect them from oxidative stress-related damage along with restoring the damaged blood vessel wall proteins (Teruo Miyazawa).

Although on an experimental basis, anthocyanins in skin care and their formulation have been found to have significant benefits in maintaining skin moisture, radiance, suppleness, and anti-ageing (Ahmed A. H. Abdellatif, 2021). The glycosidic derivatives, which are among the bioactive constituents, have been shown to have effects against thalassemia, diabetic neuropathy (DN), hyperlipidaemia, diabetes, diabetes-related inflammation, and obesity. They also have effects on AD and PD (Alzheimer's and Parkinson's diseases), CNS and CVS (central nervous and cardiovascular systems) related disorders (Khan, 2022).

As previously mentioned, anthocyanins also work to treat diseases of the eyes, such as cataracts, macular degeneration (MD), eye fatigue, and glaucoma, as well as diseases of the lungs, such as asthma, irritable bowel syndrome (IBS), and GIT (gastrointestinal tract) disorders, as well as diseases of the liver, including diseases of the kidneys and gonads, such as nephrolithiasis, urolithiasis. Anthocyanins are known as powerful nutraceuticals and are also known to stimulate the appetite and have choleretic effects. The products' bioactive components have a variety of pharmacological effects, including significant

anticancer effects, one of the most sought-after bioactivities. The cyanidin and delphinidin have been observed to inhibit epidermal growth factor receptors in cancer cells, whereas malvidin stood at lowered effectiveness against EGF receptors (Meiers, et al., 2001).

A substantial amount of research has been done and reviewed on anthocyanin's anticancer properties (Bo-Wen Lin, 2016). The effect of Cyanidin-3-Glucoside (C3G) on the inhibition of ethanol-induced activation of the ErbB2/cSrc/FAK pathway is to inhibit cell invasion and migration might be useful in preventing the spread of breast cancer brought on by ethanol. Anthocyanins' anticancer properties have been attributed to their capacity to block angiogenesis and cause apoptosis (Chang Hui, 2010). The anticancer properties of anthocyanins in Vitelotte potatoes were investigated by (Paola Bontempo, 2015). In a study by (Yi, Fischer, & Akoh, 2005) the impact of anthocyanin on the survival and apoptosis of cancer cells in muscadine grapes was evaluated. Anthocyanins in purple tea have anti-inflammatory, anticancer, and antioxidant properties.

The antibacterial properties of anthocyanins have been demonstrated in numerous investigations. Anthocyanins' capacity to cause Gram-negative bacteria's outer membrane to release polysaccharide molecules has been linked to their antimicrobial activity. Anthocyanins may also affect microbial metabolism by depriving the organism of substrates required for its growth (Elisa Pojer, 2013).

A chronic metabolic illness affecting millions of adults aged 20 to 79 is called diabetes mellitus (DM). Increased blood sugar levels brought on by poor insulin production or insulin resistance characterise this metabolic condition. Therefore, preventing excessive postprandial blood glucose rises and enhancing insulin resistance are key components of the management of DM. Anthocyanins' anti-diabetic properties have been thoroughly investigated. In streptozotocin-induced diabetic rats, petunidin-3-O-p-coumarylrutinoside-5-O-glucoside, an acylated anthocyanin, decreased fasting sugar levels (Strugała P, 2019). By lowering the glucose levels in Zucker Diabetic Fatty rats, the anthocyanin extract from mulberry fruit demonstrated strong antidiabetic activities (Sarikaphuti A, 2013).

The effect of anthocyanins in reducing insulin resistance was evaluated (Belwal T, 2017). Insulin resistance is an aberrant physiological condition where insulin from pancreatic -cells is unable to activate a signal transduction pathway in the target organs. Pancreatic-cell gene expression and insulin release were both enhanced by cyanidin. Additionally, cyanidin increased the expression of genes that may affect insulin production,

glucose homeostasis, and diabetes (Suantawee T, 2017). In db/db mice and HIT-T15 cells (ATCC CRL-1777, hamster pancreatic beta cell line), purple maize anthocyanins stimulated insulin production (Hong, 2013). Research was also done on how acetylated anthocyanins could lower of postprandial glucose levels by delaying maltase activity (Matsui T, 2002).

Some of the metabolites of anthocyanins have considerable antioxidant activity and most often play a significant part in the anthocyanins' recognised biological action. Protocatechuic acid, the main metabolic by-product of anthocyanins, has been noted for its potent antioxidant and anti-fibrotic properties linked to its ability to reduce the pathological factors that lead to liver fibrosis, such as transforming growth factor-1 (TGF-1) and connective transforming growth factor (CTGF) (Sahil Kakkar, 2014). The bioactivity of anthocyanins also includes other significant metabolites, including gallic acid, syringic acid, and 3-O-methyl gallic acid, which have been investigated for their potential as antioxidants, hepatoprotection, and anti-hepatocellular carcinoma agents (Gheena S, 2019). Numerous pre-clinical, clinical, and epidemiological research has documented the positive benefits of anthocyanins on health (Dzierżanowski, 2021) (Jian He, 2010).

2.3 INDUSTRIAL APPLICATION OF ANTHOCYANIN PIGMENT

Anthocyanins have been ingested by humans and animals for a very long period. Anthocyanins included in food have not been linked to any negative health effects when consumed orally (Jian He, 2010). It is widely accepted in Europe, Japan, the United States, and many other nations that anthocyanins derived from natural sources may be used as food colourants in foods and beverages (Nollet, 2000). The Joint FAO/WHO Expert Committee on Food Additives (JECFA) came to the conclusion that anthocyanin-containing extracts had very low toxicity based on early toxicological investigations that included mutagenicity, reproductive toxicity, teratogenicity, as well as acute and short-term toxicity evaluations (FAO/WHO, 1982). Therefore, in this present era where industries like food, medicine, and nutraceuticals are interested in the clean recovery of valuable components, the food industry has now started looking for new items, such as blue colourants, in an effort to expand the selection of natural organic colourants (colour additives of food and beverage products) (Newsome AG, 2014).

In the current era, with the increased consumer awareness of the need for healthy eating habits, the food industry has worked to develop alternative, natural sources of

chemicals that, while entirely safe, may also provide some health benefits. In light of this, the adverse health effect of synthetic food dyes, more and more food manufacturers are attempting to use anthocyanins as substitutes for FD&C red #40 (allura red, E129), the most widely used synthetic colourant (Jian He, 2010). They are safe to eat because they are present in many fruits and vegetables, including berries, beets, red cabbage, grapes, and black carrot, among others. The antioxidant and antibacterial characteristics of anthocyanins may help stabilise foods and lengthen their shelf lives. They also provide an interesting natural substitute for red/purple food colouring providing potential biological advantages (S. Silva, 2017).

Anthocyanins have filled this gap for the last three decades. They have additional health advantages, which sets them apart from other anthocyanins in the food industry. Due to their vivid colours, anthocyanins have been investigated for use as food colouring agents (Regina Cortez, 2016). Anthocyanins have also been employed in a variety of ways to produce the hues of pink, red, purple, and blue. Anthocyanins have additional health benefits that give them a unique position among anthocyanins in this sector (Alappat, 2020).

Aanthocyanin based applications are also extended to beverages, food supplements, and personal care-based products, and their industries. As a result, plants high in anthocyanins have attracted attention from the food, immune system-boosting, and medicinal use-based healthcare industries. They have also been promoted as food and drug additives with a number of other medications, herbal extracts, and the anthocyanins themselves in specific dosage forms, such as liquids, tablets, and capsules (Valentina Melini, 2019). As a result, herbal pharmaceutical preparations containing anthocyanins are widely known and regarded in society. They are also widely used by the general public, particularly through commercial websites on the Internet and as over-the-counter (OTC) medications at neighbourhood pharmacies. The market is filled with cranberry, bilberry, blackberry, blood orange, raspberry, chokeberry, elderberry, mulberry, and blueberry based products that are sold for weight loss, as antioxidants, immunostimulants, general health well-being, and for urinary tract health. The commercial products have been listed based on a number of factors, including the standard brands and their value, usefulness, certification, quantitative specifications, customer ratings, seller ranks, customer reviews, durability, and least unfavourable ratings (Khan, 2022).

New nano-formulations, synthetic products, and bioavailability enhancement strategies have led to the widespread commercialization of anthocyanin products, which have been adopted to their source plants in industrial agriculture, and crop and quality management of anthocyanin products as a marketed commodity for use by common man, elderly for age-related diseases, and adults and children as food supplements. Prospects exist in the development of synthetic analogues, formulations, and yield increases from source plants. It would also be ideal to see advancements in extraction techniques, product quantification and quality control, yield enhancements due to biotechnological breakthroughs, structure-activity connections of anthocyanin compounds, illnesses, and receptor entanglements for better therapeutic leads. It is expected that efforts will be made to satisfy future market demands through newer methods of anthocyanin product manufacture, storage, and stability (Khan, 2022).

2.4 AN OVERVIEW OF BASELLA ALBA FRUIT

The Basellaceae family includes Malabar spinach (Basella alba L., sometimes known as Basella rubra Roxb.) (Gaikwad, 2014). It is a few meters long succulent, branching, silky, twining herbaceous vine. Green or purple stems are common. The leaves are 5 to 12 cm long, stalked, fleshy, ovate or heart-shaped, tapering to a point with a cordate base. Axillary, solitary, 5-29 cm long spikes are present. Fruit is 5–6 mm long, ovoid or spherical, fleshy, stalkless, purple when mature. For therapeutic purposes, stems and leaves are vastly used (S. Sravan Kumar, 2015).

Taxonomically, Linnaeus designated the plant and distinguished two species as Basella rubra L. and Basella alba L. The differences between these two species can be seen in their stem colours and leaf features (ROSHAN ADHIKARI, 2012). Malabar spinach, Indian spinach, Ceylon spinach, climber spinach, and vine spinach are all alternative names for Basella alba L. or Basella rubra L. (Subhash Kanti Roy, 2010). According to (Saroj V, 2012), Basella alba is native to India and Indonesia and has grown there organically. Malabar spinach is a perennial vine that grows quickly and can withstand great heat; it is often grown as a cool-season food source. In summer, they abound with dark violet-blue, small stone fruits (Ajay Chaurasiya, 2021).

Different parts of B. alba are used in traditional medicine, particularly in India and China, to treat a wide range of ailments (Dennis Seyi Arokoyo, 2018). The extracts of these plants can be credited with having antibacterial, (Deka, 2017) anti-inflammatory, and depressive qualities in addition to their vitamin and mineral content. Malabar spinach contains beneficial compounds in both its stems and leaves (S. Sravan Kumar, Nutrition facts and functional attributes of foliage of Basella spp., 2015) as well as extracts from its fruits, which have been shown to, among other things, have cytotoxic properties against human cervical cancer. It has been used to cure acne and freckles in Bangladesh and India for antipruritic and burn (Agnieszka Kumorkiewicz-Jamro, 2021). The leaves and stem of B. alba have been utilized in Indian Ayurvedic medicine to cure cancers like melanoma, leukaemia, and mouth cancer (Agnieszka Kumorkiewicz, 2018).

The vegetable Basella alba is relatively inexpensive. Most population in Bangladesh do not use mature B. alba fruit (Pui fall) as food and typically discard it, whereas other communities use it to prepare a rice-and-curry dish. It's interesting to note that mature B. alba fruits have a reddish hue and can be employed in the food industry as food additives, food preservatives, and food supplements. They can also boost food texture. A potential source of natural colourant is the B. alba fruit, which has rich red-violet flesh and a dark blue exterior (Ahmad Adekilekun Tijani, 2012). The fruit offers a dark violet culinary colouring. Pastries and sweets are coloured using the bluish sap from the B. alba fruit (Begum, 2010). Because of the abrupt colour change that occurs with a change in pH value, fruit extract of the Basella alba is employed as a natural indicator in acid-base titration to identify the endpoint. The dye's initial hue is violet, and its pH level is 4.9 (Das, 2016).

Natural indicators are more cost-effective, straightforward, readily available, and environmentally friendly than the synthetic colourants typically employed in acid-base titration (Mitra A, 2016). Natural pigments derived from plants have gained a lot of interest due to their potential applications in the food and cosmetics industries, as well as in nutraceutical and pharmaceutical research due to the growing interest from health-conscious consumers and researchers (Almedia, 1996). Thus, both natural colour and food preservatives can both be derived from the B.alba fruit and employed in the food sector to improve food texture. Compared to the synthetic azo dye used in the food business, it is a safer and healthier food colour. Because of its beneficial benefits on health, Betalains isolated from Basella plant fruits are also employed in food compositions (S. Sravan Kumar

P. M., 2015). These colourants also have uses in the healthcare, cosmetics sectors and in fabric dyeing due to the presence of pigments (Khan MI, 2015).

METHODOLOGY

CHAPTER III

METHODOLOGY

The methodology of the present study entitled **"Extraction of anthocyanin from selected plant sources and its food application"** is discussed as follows:

3.1 Selection of Plant sources (samples)

3.2 Sample collection

3.3 Preparation of samples

3.4 Extraction of Anthocyanin

3.5 Total Anthocyanin content

3.6 Comparison of total anthocyanin content for further analysis

3.7 Analysis of Anthocyanin Stability

3.8 Comparison of Anthocyanin Stability

3.9 Analysis of Biological activity of the selected Anthocyanin

3.10 Application of the extracted Anthocyanin

3.1 Selection of Plant sources (samples)

The plant sources required for the study were selected based on prior studies of pigments extracted from each source with the exception of extraction of anthocyanin pigment. Selection of the samples was also based on the minimal use of the plant sources in daily life, its ease of availability and health benefits.

3.2 Sample collection

The samples were collected freshly from the plants as such and stored in aseptic conditions. These stored samples were transported to the laboratory (PVT Merit Biolabs) immediately.

3.3 Preparation of samples

The collected samples were washed using fresh water in order to remove the dirt and dust particles from the environment. This is to ensure that there's no contamination, which in turn can affect the obtained pigment extract.

3.4 Extraction of Anthocyanin

50 grams of each plant sample was weighed and transferred to a clean beaker. To this beaker containing the plant sample a prepared mixture of 500 ml of 50% acetone was added at a 1:10 ratio. This mixture is then allowed to stand for 5 hrs which is then boiled in a water bath at a temperature of 50°C for 45 minutes. The acetone mixture is stirred using a glass rod for confirming the homogeneity. This homogeneous mixture was then filtered using a normal filter paper in order to remove the suspended solid particles for the collection of the extract as such.

3.4.1 Separation of Anthocyanin

The separation of anthocyanin from solvent was performed after the extraction method. For this process, the extraction was transferred to large plates, where the initial weight of the plate when empty was noted clearly. This plate was then placed in a water bath at 60°C for 24 hours. This step ensures the removal of acetone by evaporation, leaving anthocyanin pigment alone back in the plate. After removing from the water bath, the weight of the plate after evaporation of acetone from the mixture was noted. This solid state of anthocyanin was made soluble using dimethyl sulphoxide at a 1:10 ratio of the obtained extract.

3.5 Total Anthocyanin content

The Anthocyanin content of the samples is determined according to the procedure described by Du and Francis (1973). In this method, a known volume of the filtered extract was diluted to 100 ml with the extracting solvent. The intensity of the coloured mixture was then measured at 535 nm using Spectrophotometer. The total Anthocyanin content referred to as delphinidin-3,5-subside and was calculated using the following equation:

- Total Anthocyanin (mg/100g) $= \frac{Absorbance \times Dilution\ factor \times 100}{Sample\ weight \times 55.9}$

3.6 Comparison of total anthocyanin content for further analysis

The total anthocyanin content of each sample obtained was compared. The samples that resulted in the highest anthocyanin content and highest absorbance were selected to analyse their stability.

3.7 Analysis of Anthocyanin Stability

3.7.1 Effect of pH

The effect of pH variation on the stability of anthocyanin was studied on a wide range of pH values ranging from 3-12 viz; 3, 5, 7, 9 and 12. To analyse the pH stability, 5 test tubes of 10 dilution of anthocyanin were prepared by adding 9 ml distilled water to 1 ml of anthocyanin extract. To these test tubes, suitable desirable pH was obtained by mixing with alkali, Sodium hydroxide and acidic Sulphuric acid in the pH meter reading. The degradation of anthocyanin was followed by periodic measurements of the colouring power of the samples at 520 nm in the different buffered extracts.

3.7.2 Effect of Temperature

To analyse the temperature stability, 5 test tubes of 10 dilution of anthocyanin were prepared by adding 9 ml distilled water to 1 ml of anthocyanin extract. These test tubes were marked according to the temperature they were exposed to as 2°C (Refrigeration Temperature), 37°C (Incubation Temperature), 27°C (Room Temperature), 40°C (Water bath) and 60°C (Water bath). The retention value of anthocyanin was estimated by the colouring power of the samples at 520 nm in the different extracts after exposure to the desired particular temperatures for 24 hours.

3.8 Comparison of Anthocyanin Stability

After categorizing the samples among the high content of anthocyanin, a secondary selection of samples based on higher stability in varying pH and temperature is considered. This classification led to the selection of a single anthocyanin pigment sample for further analysis and application of anthocyanin pigment.

3.9 Analysis of Biological Activity of Selected Anthocyanin

The biological activity of the selected anthocyanin pigment was studied by

3.9.1 Determination of total phenolic content

3.9.2 Determination of total flavonoid content

3.9.4 Determining total antioxidant activity

3.9.5 Quantitative Test for Free Radical Scavenging Activity by DPPH

3.9.6 Antibacterial Assay

3.9.1 Determination of total phenolic content

The amount of total phenolics in the plant sample is determined by the Folins -Ciocalteu method. For this method, a 100-dilution solution of anthocyanin was prepared. This is done by adding 9ml distilled water to 1ml of the extract, after which 1ml of this prepared dilution is further added to 9ml of distilled water. Dilution is done in order to obtain an accurate spectrophotometer reading.

To 0.5 ml of the test sample, 1.5 ml Folin-Ciocalteau reagent was added and allowed to stand for 5 minutes at 22°C. After 5 minutes, 2 ml of 7.5% of sodium carbonate was added. These mixtures were incubated for 90 min in the dark with intermittent shaking. After incubation, the development of blue colour was measured at 725nm using a UV – visible spectrophotometer. The phenolic content was calculated as gallic acid equivalents GAE/g on the basis of the standard curve of gallic acid. The result was expressed as Gallic acid equivalents (GAE/g) of the plant material.

3.9.2 Determination of total flavonoid content

The aluminium chloride method is used for flavonoid determination. In this method, Quercetin was used as standard and flavonoid content is measured as quercetin equivalent. For this method, a 100-dilution solution of anthocyanin was prepared. This is done by adding 9ml distilled water to 1ml of the extract, after which 1ml of this prepared dilution is further added to 9ml of distilled water, which is used as the anthocyanin test sample. Dilution is done in order to obtain an accurate spectrophotometer reading.

An aliquot (0.5ml) of the extract is taken and different concentrations(100 -500 microgram/ml) of standards were taken into a 10ml volumetric flask, containing 4ml distilled water and 0.3ml of 5% $NaNO_2$ were added to the flask. After 5 min, 0.3ml of 10% $AlCl_3$ was added to the mixture. At the 6^{th} min, 2ml of 1M NaOH was added and the volume was made up to 10ml with distilled water. After 15 min of incubation, the mixture is seen to be turned to pink colour which is when the absorbance is noted at 510nm using a UV-Visible spectrophotometer. Distilled water was used as blank. The TFC was expressed in mg of Quercetin equivalents per gram of extract.

3.9.3 Total Antioxidant Activity

The total antioxidant activity of the extract was determined according to the method of Prieto et al. For this method, a 10-dilution solution of anthocyanin was prepared by adding 9ml distilled water to 1ml of the anthocyanin pigment test sample. 0.3mL of each fraction was mixed with 3.0mL of reagent solution (0.6M sulphuric acid, 28mM sodium phosphate, and 4 mM ammonium molybdate). The reaction mixture was incubated at 95°C for 90 min in a water bath. The absorbance of all the sample mixtures was measured at 695 nm. Ascorbic acid (100 μg/mL) was used as standard control.

3.9.4 Quantitative Test for Free Radical Scavenging Activity by DPPH

Radical scavenging activity of the plant extracts was conducted based on the method developed by Brand-William et al. (1995) with slight modifications. A 10-dilution solution of anthocyanin was prepared by adding 9ml distilled water to 1ml of the

anthocyanin pigment, which is used as a test sample in this method. 3 Test tubes were marked as positive, and control and test were taken. To the positive test tube, 0.05g of Ascorbic acid was added to 200 µl distilled water, while to the control test tube, 200 µl of distilled water alone was added. To the test tube for the sample, marked as 'test', 200µl of the test sample was added. All the contents in the 3 test tubes were made to a total volume of 3ml by adding 2.8ml of distilled water. 3ml of DPPH solution was added to all the 3 test tubes. The reaction was allowed to take place in the dark for 30 min and the absorbance reading at λmax= 517 nm was recorded to determine the concentration of remaining DPPH.

The results were expressed as IC50 (Inhibition concentration at 50% scavenging activity). Ascorbic acid was used as standard in comparison with the extracts.

$$\% \text{ DPPH Radical scavenging activity} = \frac{Absorbance\ of\ control - Absorbance\ of\ sample}{Absorbance\ of\ control}$$

3.9.5 Antibacterial activity

The antibacterial activity of the anthocyanin test sample was tested by using well-diffusion method. In order to check the antibacterial activity 3 major food contaminating micro-organisms were selected. This includes Salmonella, Staphylococcus aureus and E. coli. Three agar petri- plates were marked accordingly for all 3 different pathogens. To each of these petri-plates, their respective micro-organisms were swabbed as per the marking. Three wells were made on each petri-plates, in which one well was marked as test control, while the rest two wells were marked as positive and negative controls. To the test control well the sample was added in such a way that it doesn't overflow, but fills the well hole. To the positive control Antibiotic Solution 100X Liquid was added in a similar way. The negative control was left as such for the growth of pathogens. After performing these steps on all three petri-plates, the Petri-plates were incubated at 37℃ for 24 hours. After incubation, the diameters of the growth inhibition zones were measured in mm using a Zone scale.

3.10 Application of the extracted Anthocyanin

3.10.1 New Product Development

There's no denying that a refreshing drink is great for cooling off in summer. The Virgin Mojito is a Cuban drink imparting a sweet citrus flavour with a minty zing. This mocktail is a hit with both kids and adults alike. At present day, a number of mojito concentrates are available in the market, where colourful ones impart a visual interest to the consumers because of their flavour from lime and their ease of use in large quantities for a crowd. Here are a few twists on the traditional Virgin Mojito which is made by using a natural colourant, anthocyanin replacing the synthetic colour for a splash of colourful refreshment.

i. Preparation of Coloured Virgin Mojito Mocktail Concentrate

- 240 g of sugar is heated in 180ml of water to make a sugar syrup for the concentrate.
- Once it becomes a homogeneous mixture keep the syrup aside for cooling.
- Squeeze the lemons in such a way that the concentrated lime extract of 120 ml is obtained.
- Mix the previously made sugar syrup and the lemon extract.
- Add 10 ml of the extracted anthocyanin pigment to impart colour to the Virgin Mojito Concentrate.
- Store the concentrate in a refrigerator for future use.

For Virgin Mojito Mocktail Drink,

- To a glass, add 4-5 mint leaves and crush them.
- Mix the crushed mint juice along with sparkling water or soda.
- Add the desired amount of concentrate to this mixture according to the desired taste.

3.10.2 Sensory evaluation of Coloured Virgin Mojito Concentrate Drink

Sensory evaluation is an analytical method in which the human senses serve as a measurement tool to determine the quality and/or to describe the condition of a food product. The sensory test was conducted based on 9 points Hedonic scale for naturally coloured concentrate of Mojito by 5-panel members.

Table 1: Sensory evaluation score sheet

Score/Rating	Hedonic scale standard
9	I like extremely
8	I like it very much
7	I like moderately
6	I like slightly
5	I neither like or dislike
4	I dislike slightly
3	I dislike moderately
2	I dislike it very much
1	I dislike extremely

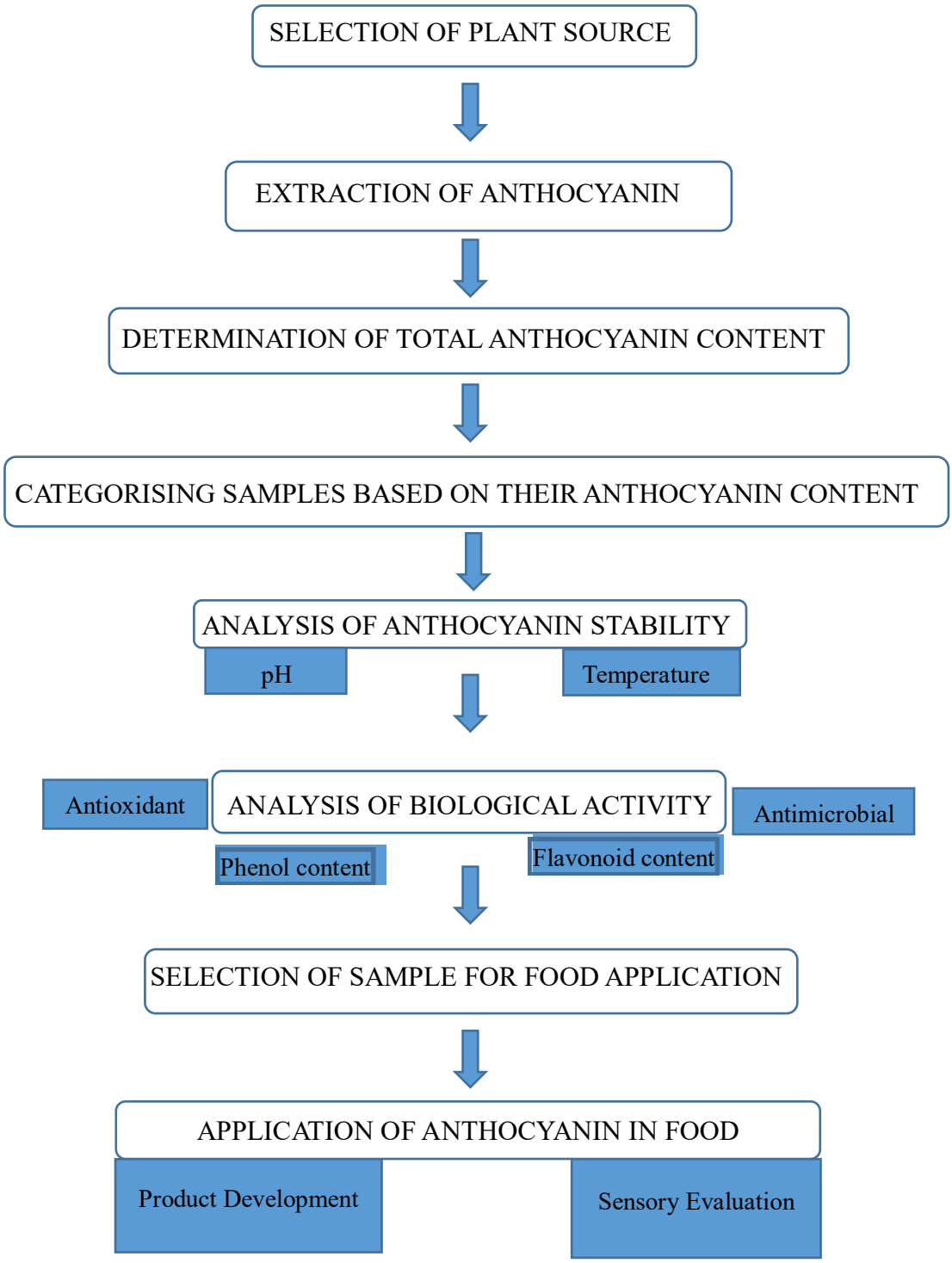


Figure 1: Research Design

RESULT AND DISCUSSION

CHAPTER IV

RESULT AND DISCUSSION

The result and discussion of the study entitled **"Extraction of anthocyanin from selected plant sources and its food application"** is discussed under the following heads:

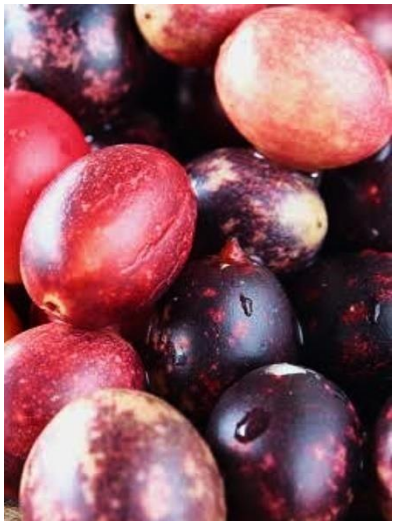
4.1 Selection of Plant Sources

The plant sample was collected directly from natural plants. The criteria used for the choice of samples are: it should be able to provide colour, the sample should be non-toxic in nature, the sample must be edible, should provide health benefits. Based on the above-mentioned criteria, the following plant sources were used for the present study:

i. Basella alba (Malabar Spinach) fruit
ii. Carissa carandas (Karonda) fruit
iii. Celosia cristata (Cockscomb)
iv. Dregon fruit peel
v. Banana bract

Plate 1: Selected plant sources for Anthocyanin extraction

Sample 1
Basella alba fruit
(Malabar spinach fruit)



Sample 2
Carissa carandas fruit
(Karonda fruit)

Sample 3
Celosia cristata
(Cockscomb)

Sample 4
Dragon fruit peel

Sample 5
Banana bract

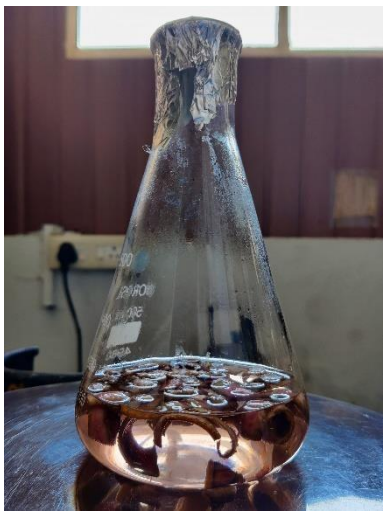
4.2 Extraction of anthocyanin

Anthocyanin Extraction for all 5 samples was done by soaking the samples in 50% acetone and keeping them in water bath for a few hours. The acetone water bath helps in extracting all the pigments as much as possible as acetone is a good solvent that is been chemically used in food laboratories. This mixture is then filtered using filter paper. The acetone is evaporated for the separation of the anthocyanin extract. The amount of extract left in the plate in dry solid form from 50g of each sample is then stored as a solution by mixing the solid form with dimethyl sulphoxide at a 1:10 ratio.

Sample 1

Sample 2

Sample 3



Sample 4

Sample 5

Plate 2: Soaking of Plant sources samples in acetone for complete extraction of anthocyanin

Plate 3:Filtration of the mixture for separating the solid particles

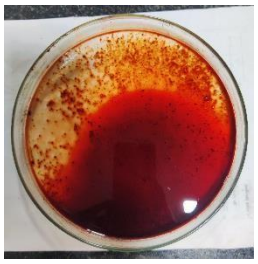
Sample 1

Sample 2

Sample 3

Sample 4

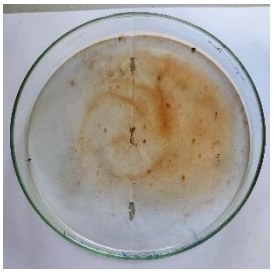
Sample 5



Sample 1

Sample 2

Sample 3



Sample 4

Sample 5

Plate 4: Anthocyanin after evaporation of acetone in water-bath

4.3 Total anthocyanin content

The total Anthocyanin content referred to as delphinidin-3,5-subside and was calculated using the following equation for all 5 samples:

□ Total Anthocyanin (mg/100g) = $\frac{Absorbance \times Dilution\ factor \times 100}{Sample\ weight \times 55.9}$

Therefore, the Absorbance of the extracted pigment at 535nm, dilution factor and sample weight was noted to obtain the total anthocyanin content in each plant sample.

Table 2 : Absorbance and dilution of the anthocyanin sample for UV Spectrophotometer reading

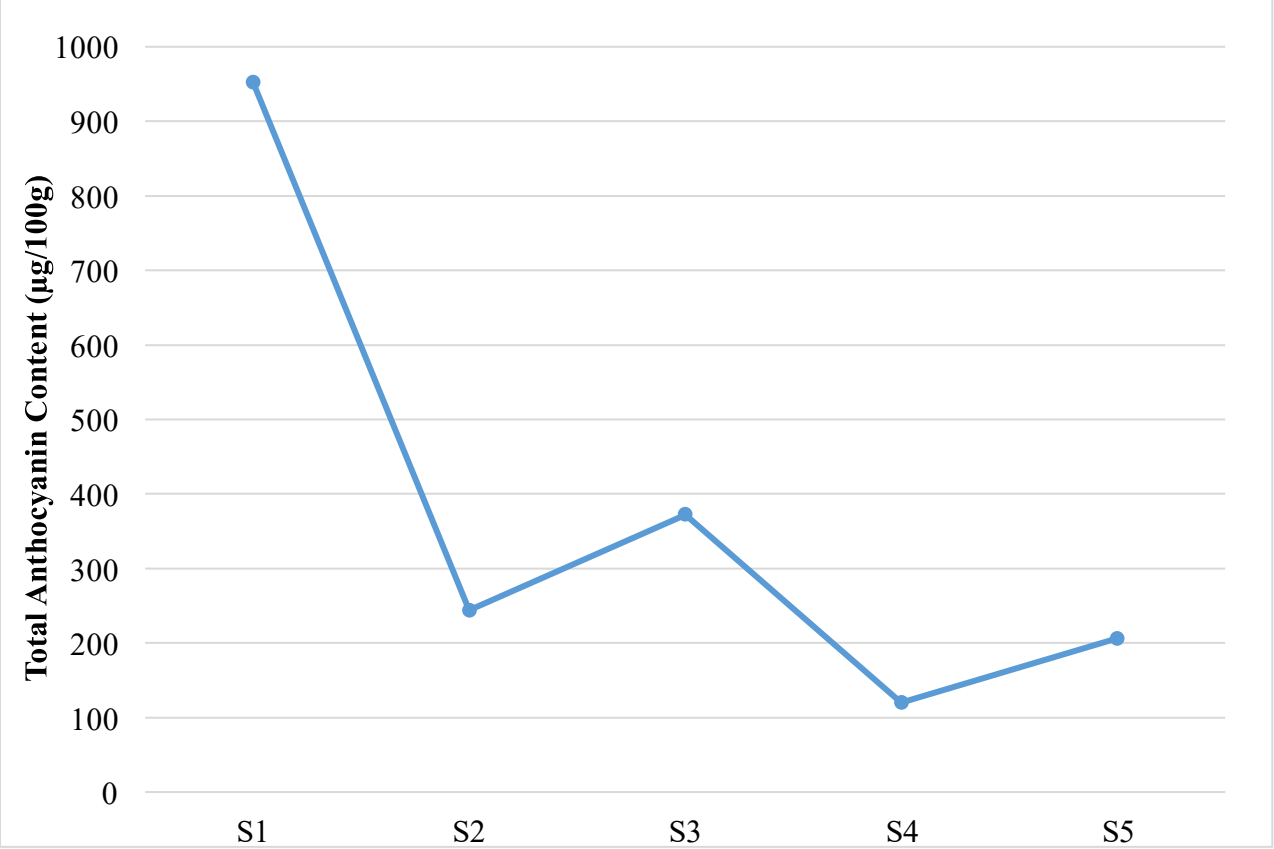
Sample No.	Absorbance at 535nm	Dilution Factor	Sample weight
Sample 1	1.311	400	1
Sample 2	1.644	400	4.83
Sample 3	2.078	60	0.6
Sample 4	0.686	40	0.41
Sample 5	1.127	60	0.56

By substituting these values to the equation, the total anthocyanin content of each sample per 100g are listed in Table 3.

Table 3 : Total Anthocyanin content of different plant sources

Sample No.	Sample Name	Total Anthocyanin (µg/100g)
Sample 1	Basella alba fruit	952
Sample 2	Carissa carandas	244
Sample 3	Celosia cristata	372
Sample 4	Dragon fruit peel	120
Sample 5	Banana bract	206

Figure 2: Total Anthocyanin content per 100g in selected plant sources



The highest content of Anthocyanin pigment was for Basella alba fruit (952 µg/100g) which is followed by Celosia cristata (372µg/100g), Carissa carandas (244µg/100g), Banana bract (206µg/100g) and the least in Dragon fruit peel (120µg/100).

4.4 Comparison of total anthocyanin content and absorbance at 535nm

From the obtained result it was observed that Basella alba fruit had the highest content of anthocyanin compared to all the anthocyanin obtained among the 5 different plant samples. But the absorbance value was the highest for Carissa carandas fruit. Therefore, these two samples with the highest anthocyanin content and highest absorbance at 535nm UV- Spectrophotometry was subjected to various temperatures and pH to check their stability and for the ultimate selection.

Figure 3 : Total Anthocyanin content for comparative study

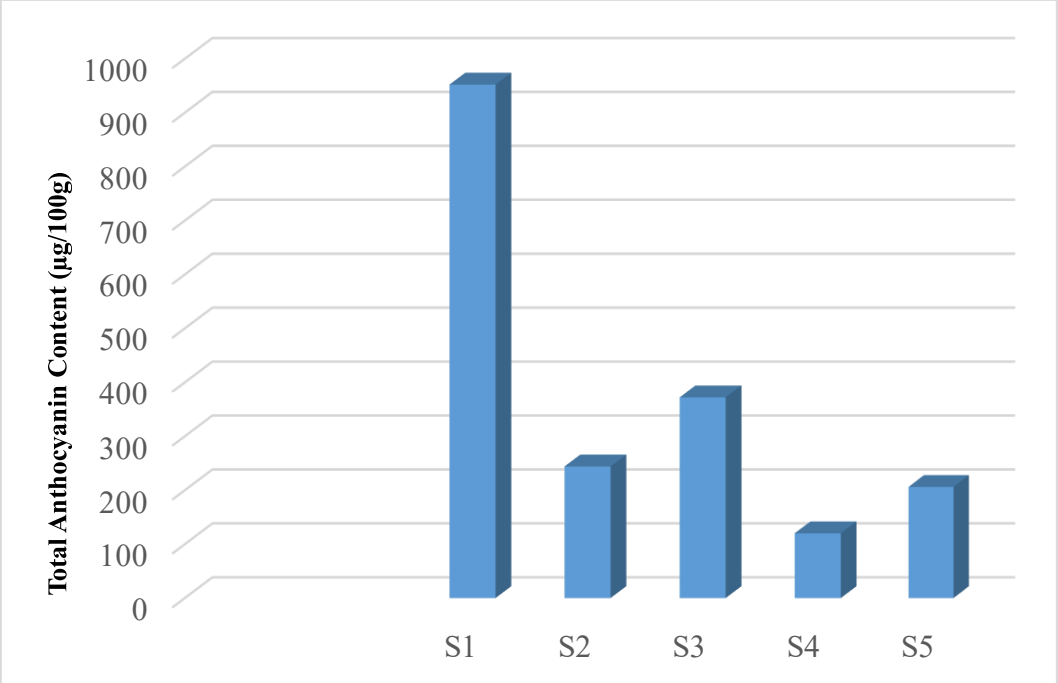
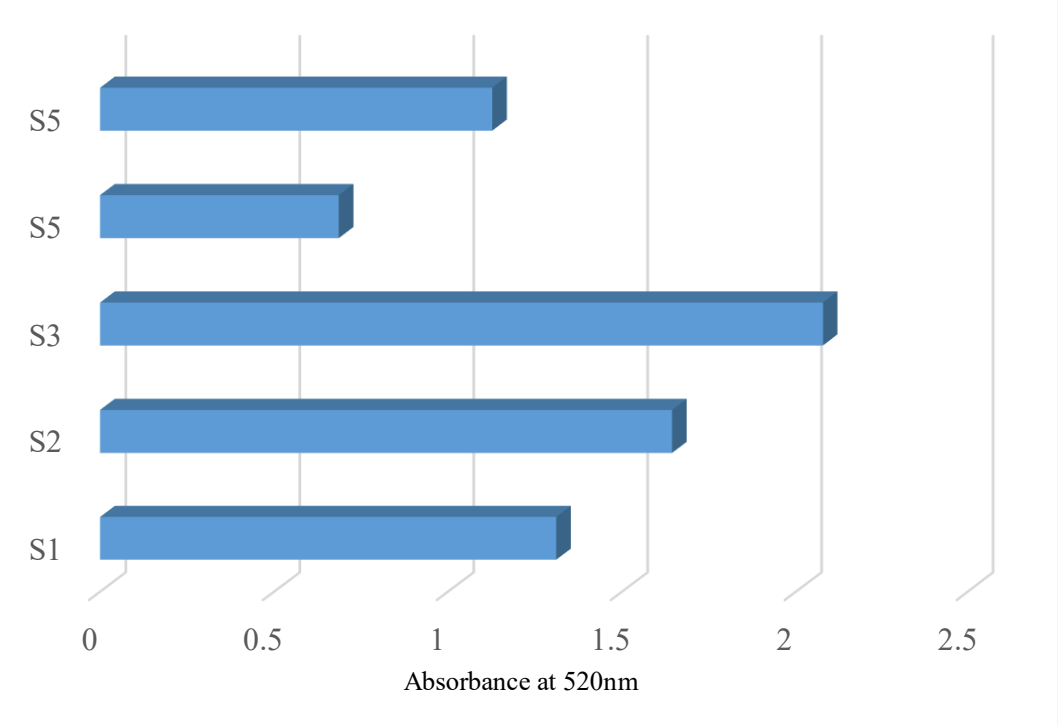


Figure 4 : Absorbance of anthocyanin pigment at 520nm



4.5 Analysis of Anthocyanin Stability in Various pH

As we know from various studies Anthocyanins are unstable at certain pH, in the present study the anthocyanin obtained from Basella alba fruit (S1) and Carissa carandas (S2) at a 10 dilution were subjected to various pH ranging from acidic pH of 3 to alkali pH of 12 to study the highest stable pH for Anthocyanin.

The result obtained by Colorimeter reading at 520 nm is shown in Table 4.

Plate 5: Basella alba fruit Anthocyanin Pigment stability in varying pH

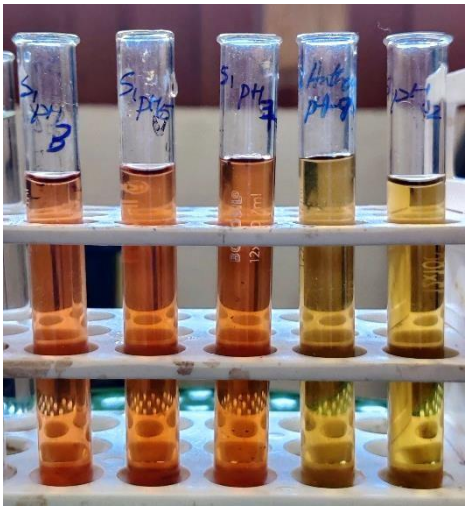


Table 4 : Colorimeter Reading for Basella alba fruit Anthocyanin in various pH at 520nm

pH Value	Colorimeter Reading at 520nm
3	0.89
5	1.23
7	1.15
9	0.84
12	0.59

Figure 5: Stability of Basella alba fruit Anthocyanin in varying pH

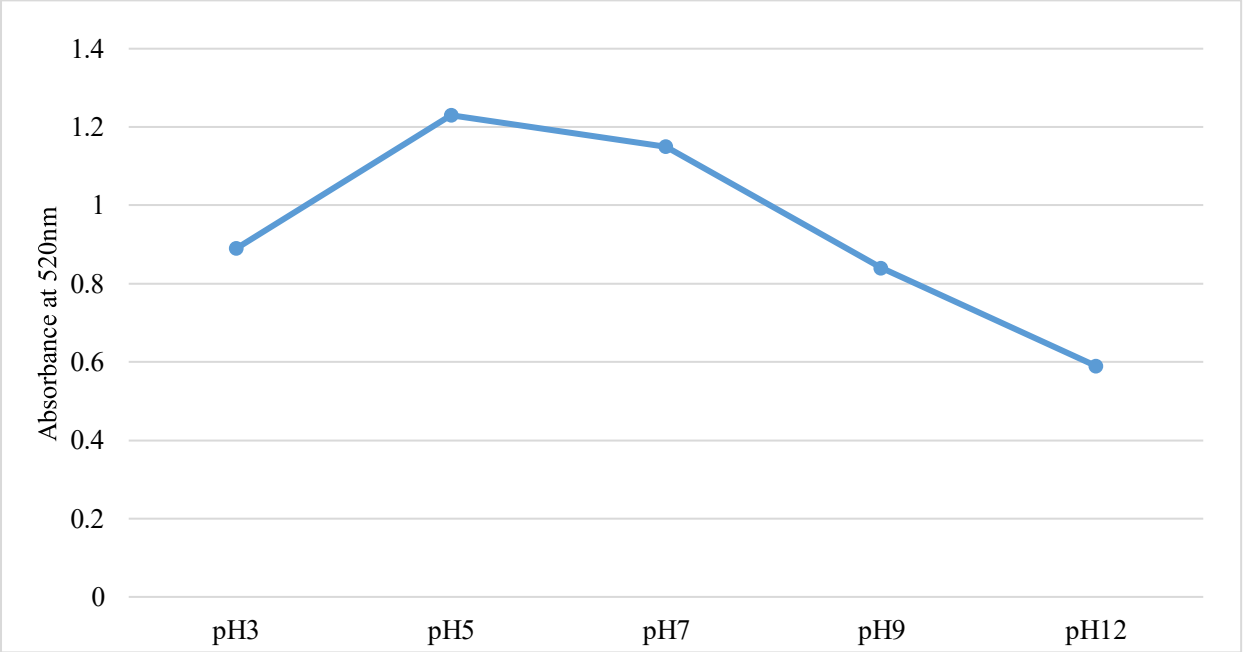


Plate 6: Carissa carandas Anthocyanin Pigment stability in varying pH

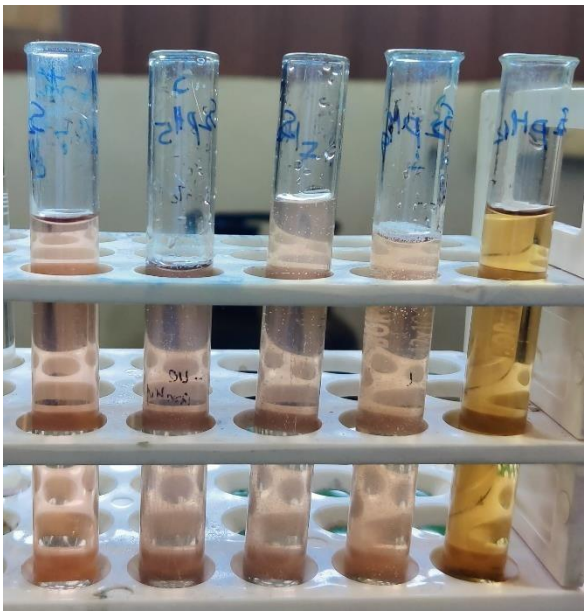
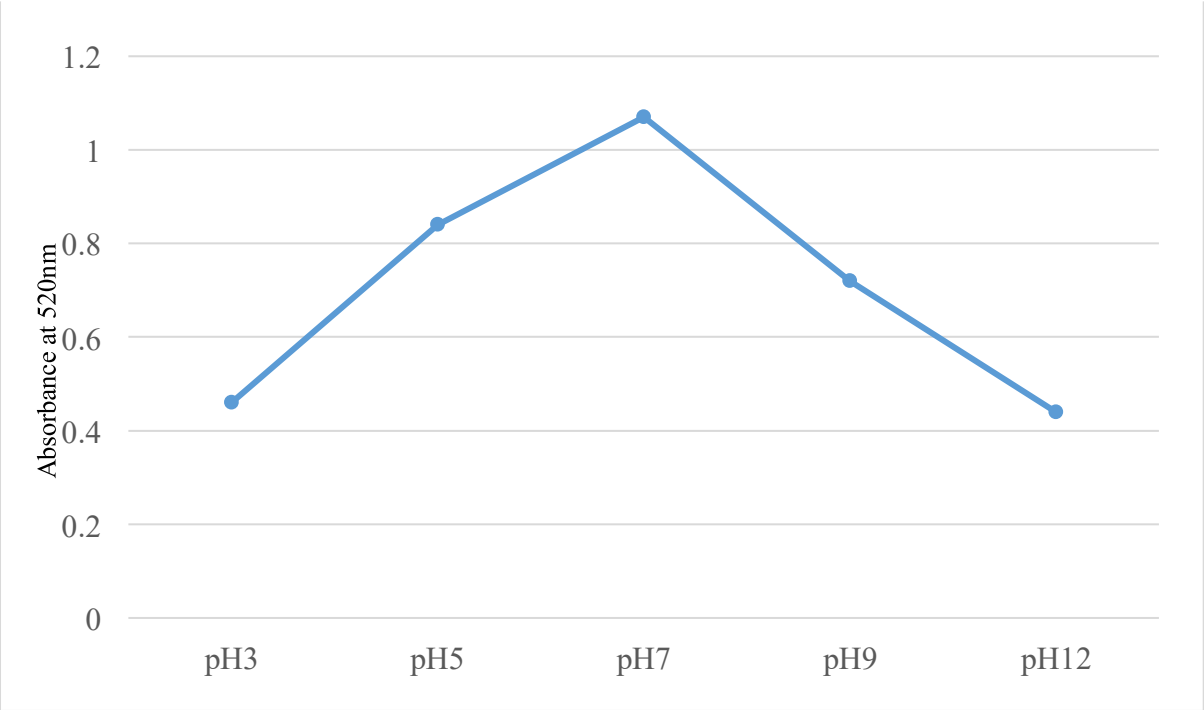


Table 5 : Colorimeter Reading for Carissa carandas Anthocyanin in various pH at 520nm

pH Value	Colorimeter Reading at 520nm
3	0.46
5	0.84
7	1.07
9	0.72
12	0.44

Figure 6 : Stability of Carissa carandas fruit Anthocyanin in varying pH



Hence, from these values, it is evident that the Anthocyanin pigment from S1 is highly stable at a pH of 5 while the Anthocyanin of S2 is at a neutral pH of 7. But considering a commonness among the pH values, a pH of 7 keeps both the Anthocyanin pigment in the maximum possible stable condition.

4.6 Analysis of Anthocyanin stability at various temperature

The stability of anthocyanin at various temperatures was analysed to study the tolerance of the pigment when being stored at extreme conditions. 10 dilution solution of Basella alba fruit Anthocyanin and Carissa carandas Anthocyanin were subjected to 2°C, 37°C,27°C, 40°C and 60°C for 24 hours. The retention value of Anthocyanin estimated by colouring power at 520 nm is noted in Table 3 and Table 4.

Plate 7 : Stability of Basella alba fruit Anthocyanin in varying temperature

Table 6 : Colorimeter Reading of Basella alba fruit Anthocyanin in different temperatures at 520nm

Temperatures	Colorimeter Reading
2°C	1.37
37°C	1.42
40°C	1.42
27°C	0.89
60°C	0.92

Figure 7 : Stability of Basella alba fruit Anthocyanin in varying Temperature condition

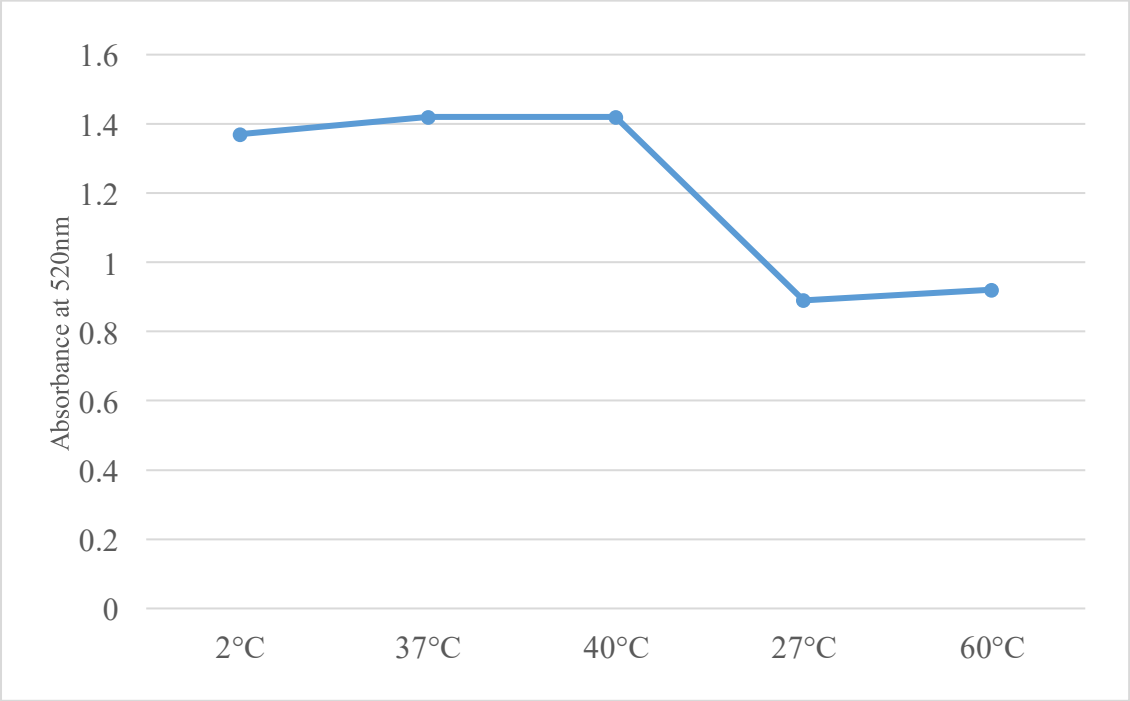


Plate 8 : Stability of Carissa carandas fruit Anthocyanin in varying temperature

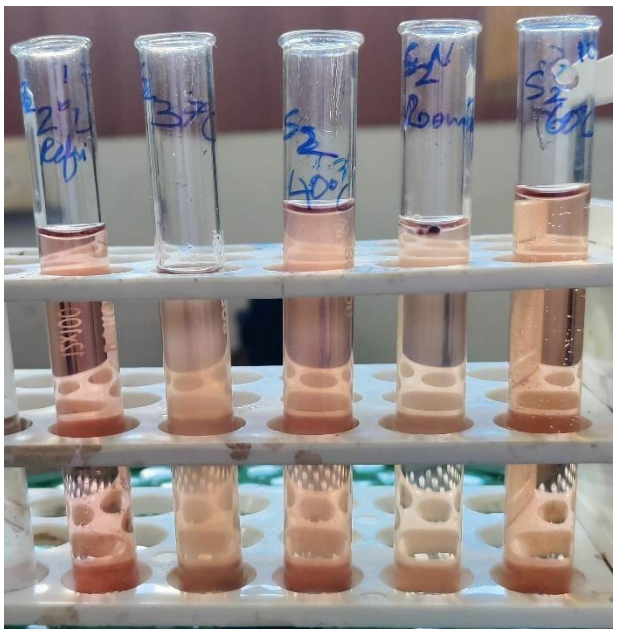
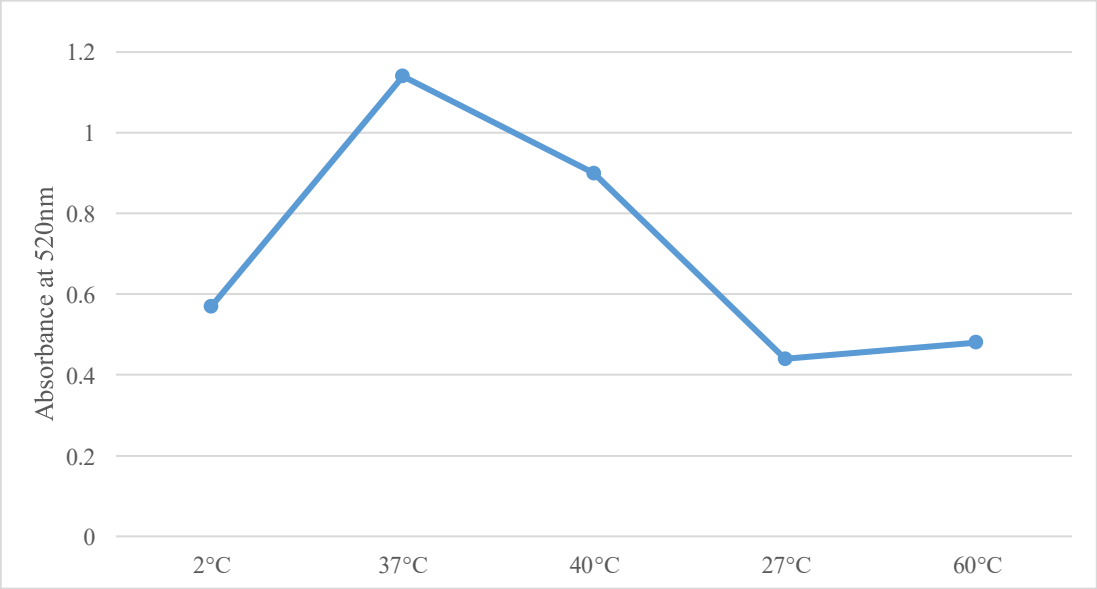


Table 7 : Colorimeter Reading of Carissa carandas Anthocyanin in different temperatures at 520nm

Temperature	Colorimeter Reading
2°C	0.57
37°C	1.14
40°C	0.90
27°C	0.44
60°C	0.48

Figure 8 : Stability of Carissa carandas Anthocyanin in varying Temperature condition



From Table 6 and Table 7, the stability of Anthocyanin pigment from both S1 and S2 is observed to be highly stable at 37°C. The colouring power seems to diminish at higher temperatures than at lower temperatures in both samples.

4.7 Comparison of Anthocyanin Stability

The temperature and pH stability study among the Anthocyanin obtained from 2 samples, namely Basella alba fruit and Carissa calendars were chosen as a criterion to continue the current study to analyse the biological properties of Anthocyanin extract.

Figure 8 and Figure 9 obtained from the prior results clearly states that Basella alba fruit Anthocyanin is the most stable Anthocyanin among the chosen plant sources.

Figure 9: Comparison of pH stability of Basella alba fruit (S1) Anthocyanin with Carissa carandas (S2) Anthocyanin

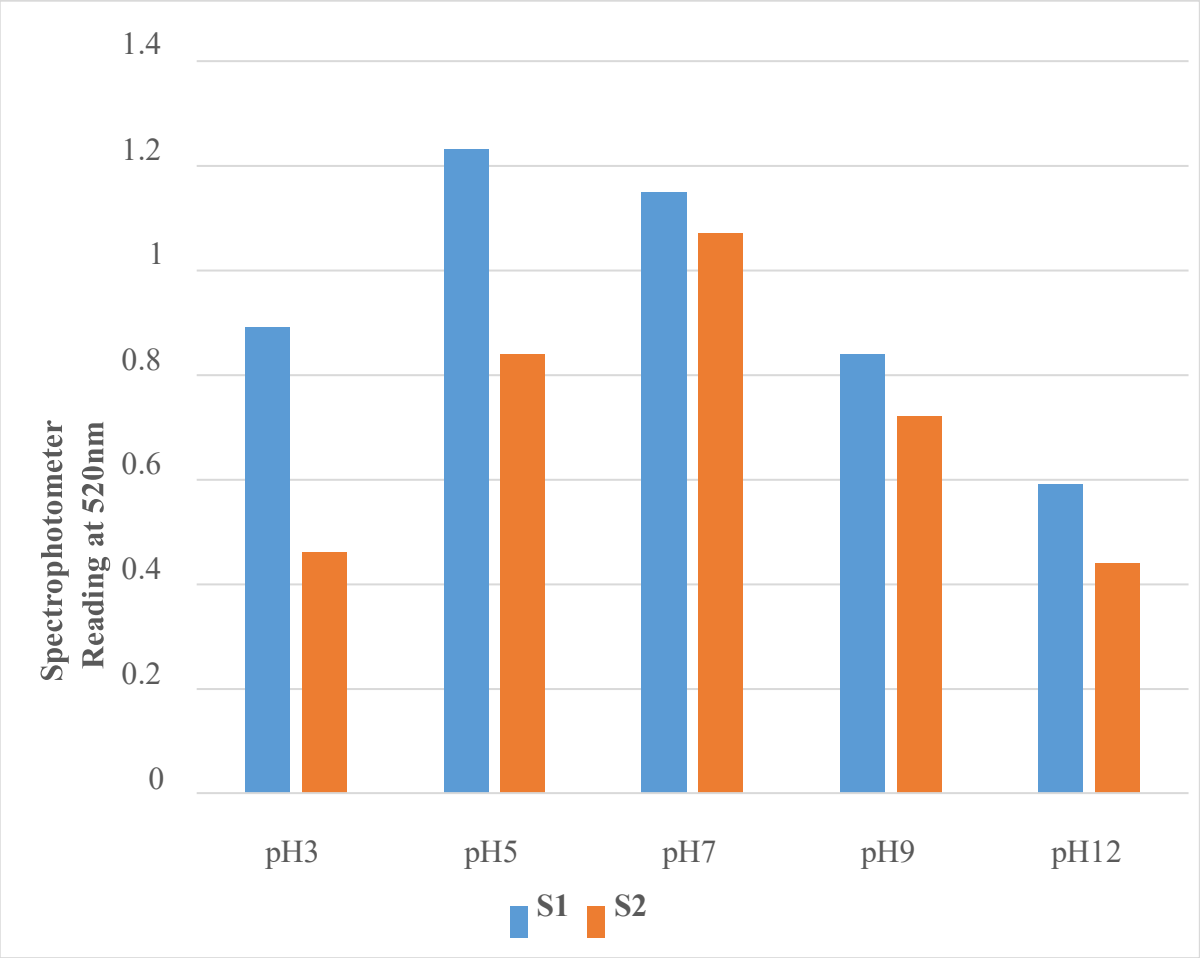
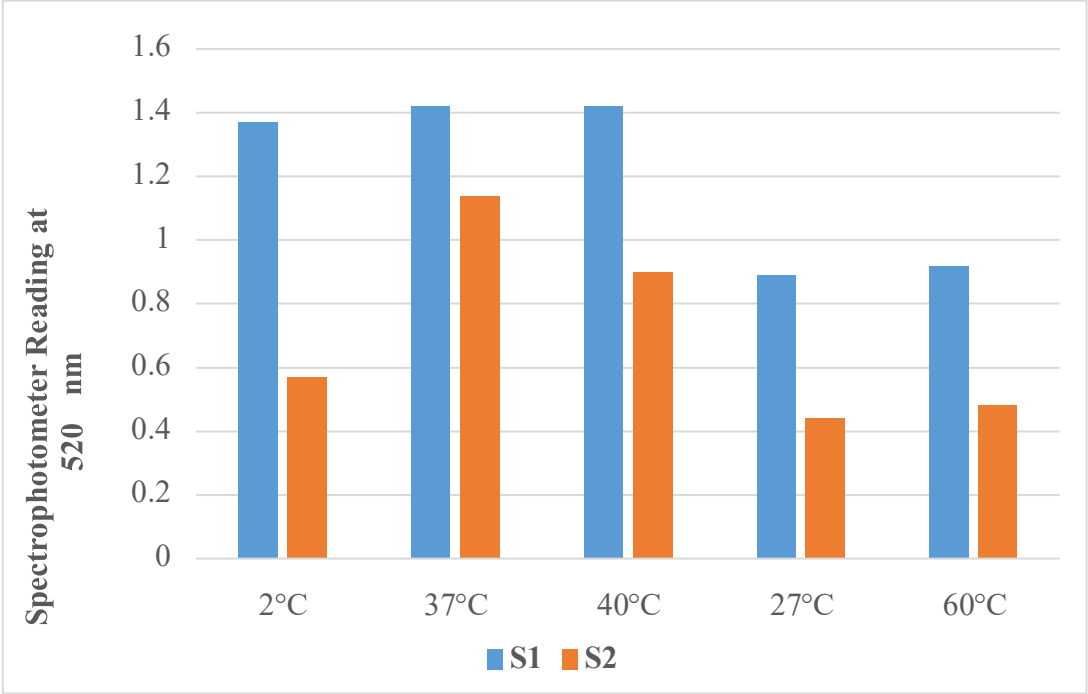


Figure 10: Comparison of temperature stability of Basella alba fruit (S1) Anthocyanin with Carissa carandas (S2) Anthocyanin



From both the comparison graphs S1 is seen to be more stable than S2 in both pH and temperature conditions. Thus, it is proved that the Total Anthocyanin content determines the stability of Anthocyanin pigment than the absorbance rate of Anthocyanin pigment. Therefore considering S1, which is Basella alba fruit Anthocyanin is the most stable Anthocyanin, further biological assay was carried out by Basella alba fruit Anthocyanins.

4.8 Analysis of Biological Activity

Analysis of Biological activity was carried out in Basella alba fruit Anthocyanin in order to know various properties that can be imparted by the pigment during its application.

4.8.1 Determination of total phenolic content of the selected Anthocyanin

The sample solution with 0.5ml of Anthocyanin and 1.5ml of Folin-Ciocalteu reagent with 2ml of 7.5% Sodium carbonate after 90 minutes of dark incubation was turned to blue colour. UV- Spectrophotometer reading of this solution was noted. This result was expressed as the Gallic acid equivalent of plant material based on a standard curve of gallic acid as (GAE/g).

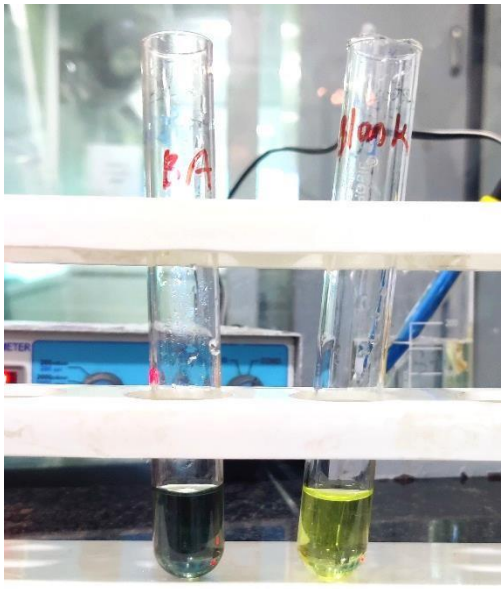


Plate 9: Anthocyanin sample with Folin-Ciocalteu reagent before incubation

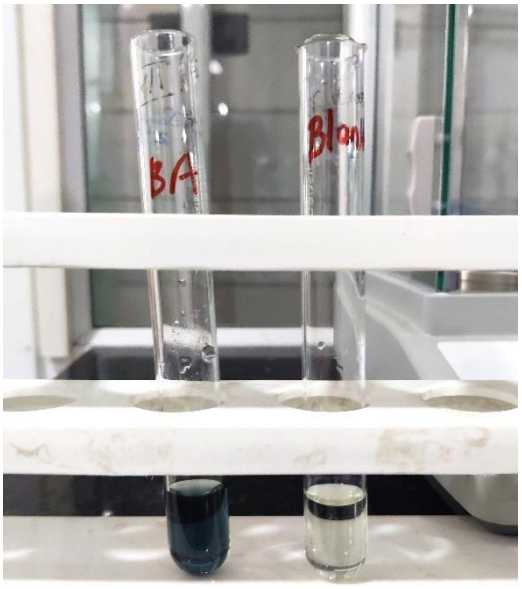
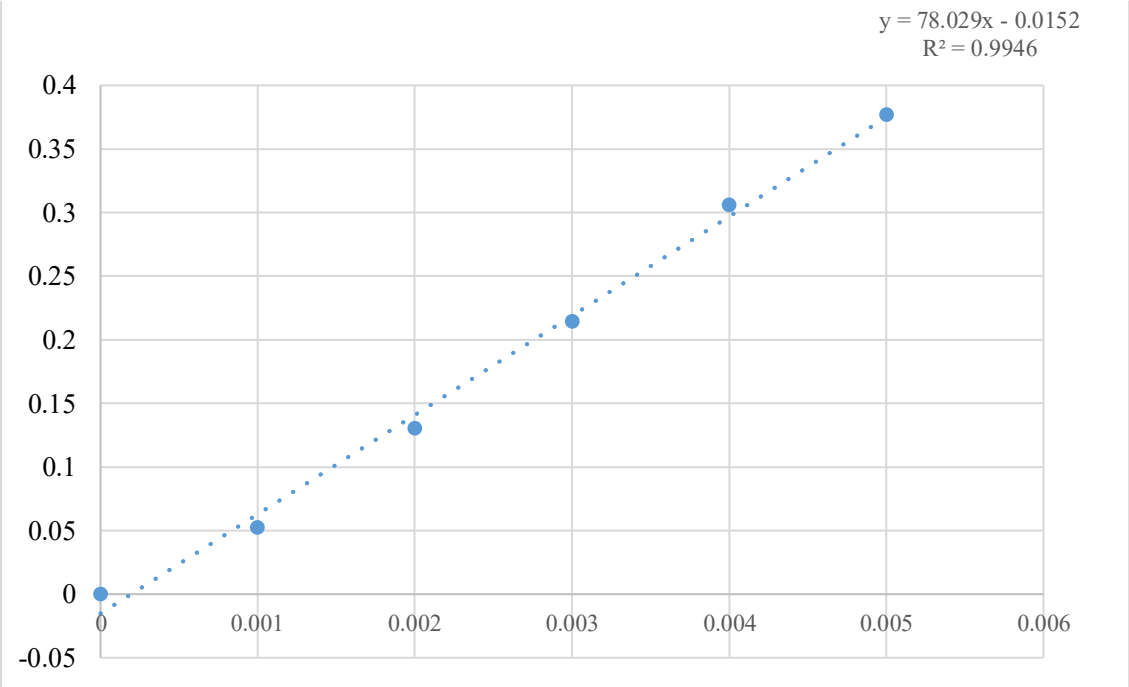


Plate 10: Anthocyanin sample with Folin-Ciocalteu reagent after incubation

Figure 11: Phenol content for standard gallic acid



The absorbance at 725nm using a UV- Spectrophotometer is **0.954**.

By using the equation obtained by Gallic acid equivalents from the graph as Phenol standard from Figure 10, which is

y=78.029x-0.0152
$R^2 = 0.9946$

The total Phenol content present in 100ml of Basella alba fruit Anthocyanin is obtained as **120mg GAE/g**

Phenol content in Basella alba fruit Anthocyanin represent that the pigment has the potential for human physiological response being anti-inflammatory, anti-proliferative and anti-ageing

4.8.2 Determination of total flavonoid content of the selected Anthocyanin

The reaction sample solution prepared to acquire total flavonoid content in Basella alba fruit Anthocyanin pigment turns to a light pink colour. The absorbance of this incubation mixture is noted at 510nm using a UV- Visible Spectrophotometer, where distilled water is placed as blank. The total flavonoid content in the Anthocyanin pigment is expressed in mg of Quercetin equivalents per gram of extract.

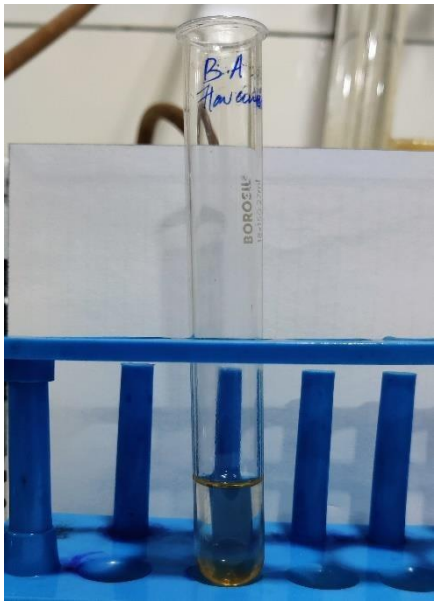
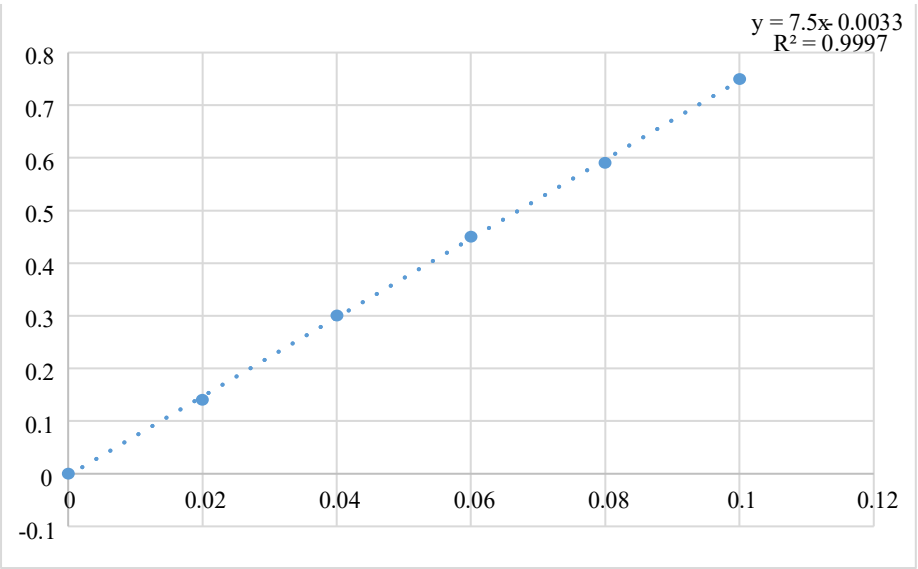


Plate 11: Light pink colour reaction sample for flavonoid determination

Figure 12: Standard Quercetin Flavonoid Content



The absorbance at 510nm using UV- Spectrophotometer is **0.073**

By using the equation obtained by Quercetin dissolved methanol equivalents from the graph as Flavonoid Standard from Figure 12

$$y=7.5x-0.0033$$
$$R^2 = 0.9997$$

The total Flavonoid content present in 100ml of Basella alba fruit Anthocyanin is **100mg QE/g**

Therefore, the Basella alba fruit Anthocyanin is said to exhibit good result in antioxidant activity based on their ability to donate hydrogen atoms to free radicals for deactivating free radicals

4.8.3 Determination of the total antioxidant activity of the selected Anthocyanin

The reaction mixture prepared for the antioxidant assay of Basella alba fruit Anthocyanin turned to dark green colour while the blank solution mixture without Anthocyanin pigment remained yellow after incubating at 95°C for 90 minutes in a water bath. The absorbance of the mixture was measured at 695nm, where Ascorbic acid (100µg/ml) was used as standard control.

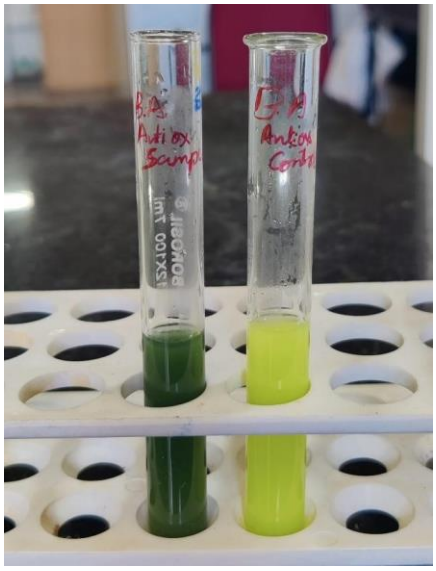
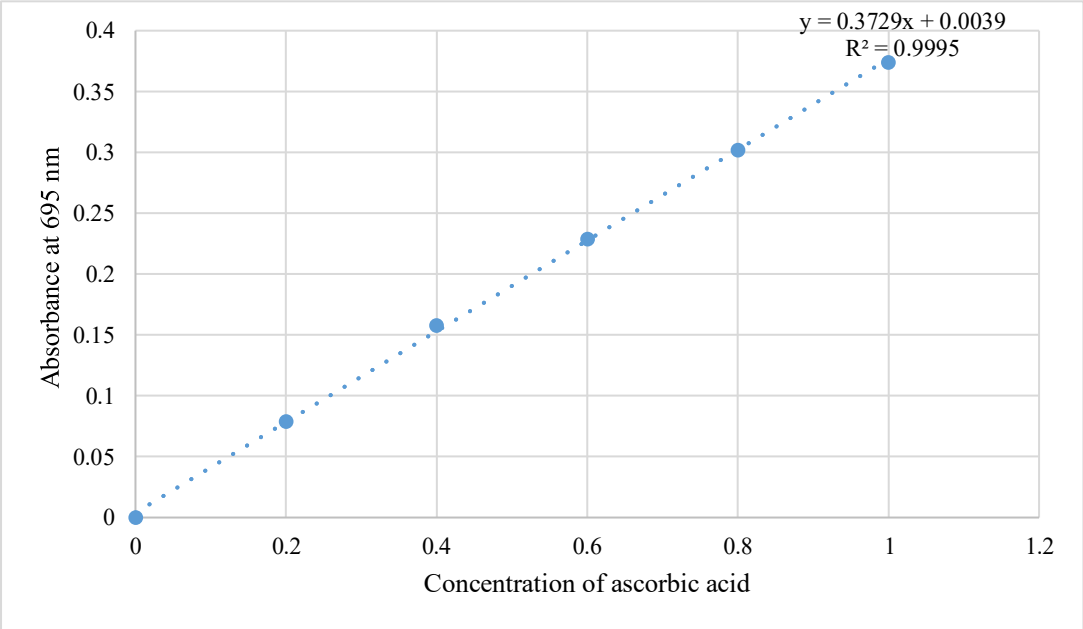


Plate 12: Reaction mixture prepared for antioxidant assay of Basella alba fruit Anthocyanin

Figure 13: Standard graph of antioxidant activity



The absorbance at 695 nm is **0.39**

By using the equation obtained by the standard graph of Antioxidant Activity

$$y=0.3729x+0.0039$$

$$R^2 = 0.9995$$

The total Antioxidant Activity of 100m of Basella alba fruit Anthocyanin is **103mg AAE/ml** compared to Ascorbic acid standards.

4.8.4 Quantitative Test for Free Radical Scavenging Activity by DPPH

The DPPH assay was included as it is a very simple test system which gives a first indication of radical scavenging potential but a secondary analysis of the antioxidant property of the test sample. (Gerhäuser, 2009). The prepared mixture of positive control, negative control and test sample was allowed to react in the dark for 30 minutes. After 30 minutes the absorbance reading at λmax= 517 nm to determine the concentration of remaining DPPH.

The results were expressed as IC50 (Inhibition concentration at 50% scavenging activity).

Ascorbic acid used as standards in comparison with the extracts.

% DPPH Radical scavenging activity = $\frac{Absorbance\ of\ control - Absorbance\ of\ sample}{Absorbance\ of\ control}$

Plate 13 : DPPH Positive, DPPH control and DPPH Sample test tube for Free Radical Scavenging analysis

Table 8 : Absorbance reading at λmax= 517 nm for DPPH

DPPH activity	Absorbance at 517nm
DPPH control	1.49
DPPH Positive	0.16
DPPH Anthocyanin Pigment	0.46

The % DPPH Radical scavenging activity of Basella alba fruit Anthocyanin is **69.1%,** which is near to DPPH positive Free Radical Scavenging property which is 89.2%. Therefore, Anthocyanin is a rich source of antioxidants.

4.8.5 Antibacterial activity

The diameter of the growth inhibition zone in agar plates was measured to study the antibacterial property of Basella alba fruit Anthocyanin. Three primary foodborne disease-causing microorganisms such as Salmonella, Staphylococcus aureus and E.coli were selected for antibacterial assay using the well diffusion method.

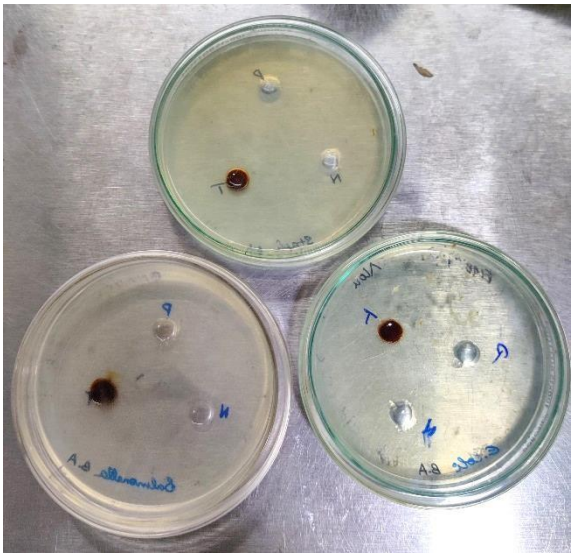


Plate 14 : Agar plates for antibacterial assay of pigment before incubation

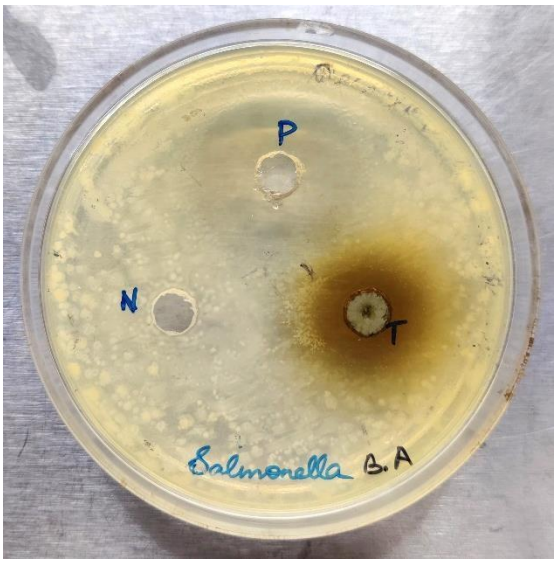


Plate 15 : Agar plates of Salmonella for zone measurement after incubating

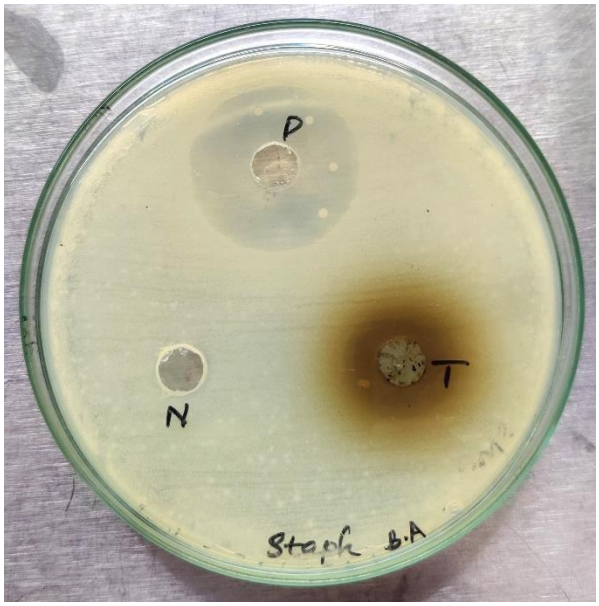


Plate 16 : Agar plates of Staphylococcus aureus for zone measurement after incubating

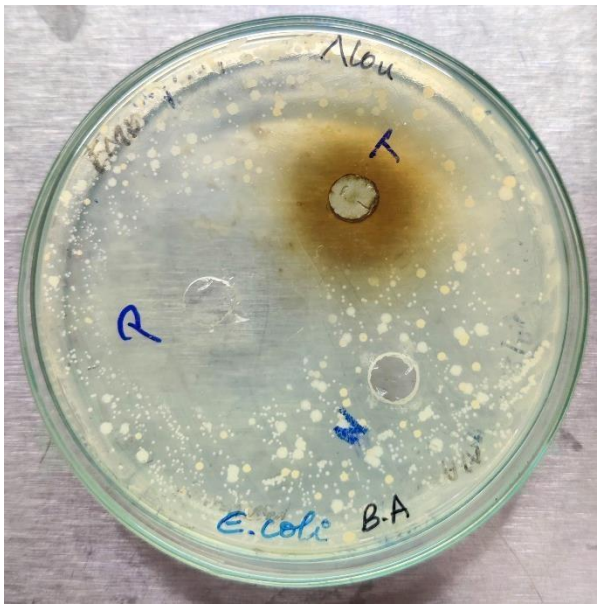


Plate 17 : Agar plates of E.coli for zone measurement incubating

Table 9: Diameter of growth inhibition zones of microorganisms by Basella alba fruit Anthocyanin

Test Organism	Growth inhibition zone of positive control	Growth inhibition zone of the Test sample
Salmonella	32mm	17mm
Staphylococcus aureus	29mm	16mm
E.coli	33mm	23mm

From the above interpretation, it is clear that Basella alba fruit Anthocyanin exhibits antibacterial properties. The highest resistance activity was on inhibiting the growth of **E.coli.**

The antibacterial property is then followed by Staphylococcus aureus and Salmonella.

4.9 Application of the extracted Anthocyanin

The Anthocyanin pigment of Basella alba fruit, which has both antibacterial and antioxidant properties is incorporated into food products as a naturally obtained food colourant. For new product development, Home- Made Virgin Mojito Concentrate was chosen in which one sample was incorporated with Basella alba fruit Anthocyanin while the other sample was left remaining in its natural colour during preparation

4.9.1 Preparation of Coloured Virgin Mojito Mocktail Concentrate

To colourless Virgin Mojito concentrate, 2.5ml of Basella alba fruit Anthocyanin was initially added and mixed thoroughly. The procedure was repeated another 3 times at a 5 mi5-minuteerval by adding 2.5ml. Totally 10ml of Basella alba fruit Anthocyanin is added to 300ml of Virgin Mojito Concentrate to impart an intense colour, as the concentrate is diluted for consumption as Mocktail.

Plate 18: Natural Virgin Mojito Concentrate

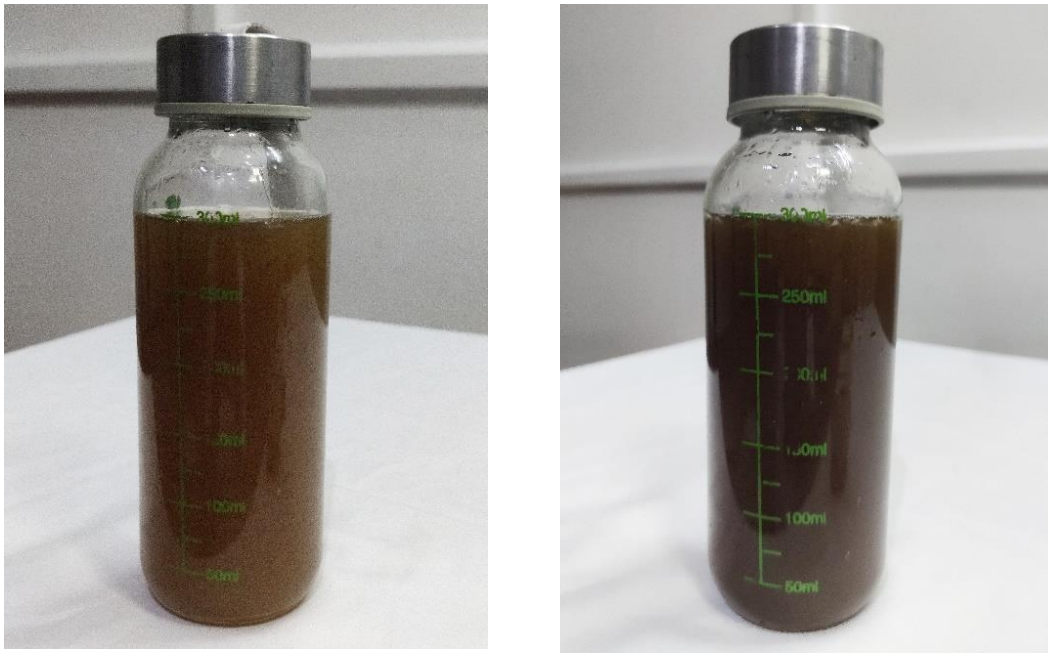


Plate 19 : Transition of Natural Virgin Mojito Concentrate to Colourful Virgin Mojito Concentrate

Plate 20 : Basella alba fruit Anthocyanin Incorporated Virgin Mojito Concentrate

4.9.2 Sensory Evaluation of Coloured Virgin Mojito Concentrate Drink

Various sensory characteristics like appearance, colour, texture, taste, flavour and overall acceptability of the standardized bread were analysed. The following table provides the average score of each the Coloured Virgin Mojito Drink with respect to these characteristics as judged by a panel of 5 ranging from kids to adults. The sensory test was conducted based on 9 point Hedonic scale for naturally coloured concentrate of Virgin Mojito by 5-panel members.

Table 10 : Sensory evaluation scores of newly developed food product using Basella alba fruit Anthocyanin

Food Product	Appearance	Colour	Odour	Taste	Overall acceptability	Mean score	%
Non-coloured Virgin Mojito	8	8	9	9	8	8.4	93.3
Coloured Virgin Mojito	9	9	8	9	9	8.8	97.7

Plate 21: Virgin Mojito in its natural colour and Basella alba fruit Anthocyanin incorporated Virgin Mojito

From the sensory evaluation conducted Coloured Virgin Mojito Drink scored higher than the Non-coloured version of Virgin Mojito Drink. Thus, Basella alba Anthocyanins are an excellent natural food colourant attributing health benefits.

SUMMARY AND CONCLUSION

CHAPTER V
SUMMARY AND CONCLUSION

The present study entitled **"Extraction of Anthocyanin from selected Plant sources and its Food Application"** aims at the development of indigenous value-added products from Basella alba fruit Anthocyanin, which is widely acceptable to a large population.

Basella alba, commonly known as watery rose apple, is from the Basella genus belonging to the Basellaceae family. Basella alba is an important green leafy vegetable and it is commonly found in tropical regions of the world. Basella alba is commonly known as Malabar spinach. Interestingly, the mature Basella alba fruits are purplish, which can be used in food industries as food additives to increase the texture of the foods as well as food preservatives and food supplements. Fruits are appealing and nutritive because of their colour, shape, unique taste and smell, enriched minerals, vitamins, and other nutrients.

There are very limited studies done on the Anthocyanin pigments of Basella alba fruit in Kerala. The objective of the study aims at assessing the physical properties, antimicrobial and antioxidant properties of the Basella alba species and to standardize value-added products from this underutilized fruit.

For the study, the Malabar Spinach fruits were collected from the home and the Fruit was subjected to extraction and separation of Anthocyanin extract. This Anthocyanin extract obtained was weighed to know the Total Anthocyanin content in the Basella alba fruit. This Anthocyanin extract was then subjected to testing their stability in different temperatures and pH, for quantitative analysis of different nutrients and phytochemicals, antioxidants and also for testing of antimicrobial properties. Additionally, value-added products were also prepared and a sensory analysis was conducted for the product to understand its acceptability among consumers.

The salient findings obtained during the course of the study have been summarized in the following points:

- 5 different plant sources were selected for the study that were underutilized by people but are edible have health benefits and are capable of producing coloured pigments in Kerala. Thus, Basella alba fruit, Karonda fruit, Cockscomb, Dragon fruit peel and Banana bract were chosen.
- Total Anthocyanin content from 5 different plant sources was assessed for a comparative study. The Basella alba fruit had the highest content of Anthocyanin pigment (952 mg/100g) which is followed by Cockscomb (372mg/100g), Karonda fruit (244mg/100g), Banana bract (206mg/100g) and the least in Dragon fruit peel (120mg/100).
- Colour retention stability of the 2 plant samples, namely Basella alba and Karonda fruit were also analysed in this study. Both the Anthocyanin pigments had their highest stability at 37°C in temperature stability analysis. However, stability analysis in acidic and alkali pH showed that Basella alba fruit Anthocyanin had the highest stability in a slightly acidic pH of 5, while Karonda fruit Anthocyanin had the highest stability in neutral pH.
- The total phenolic content of Basella alba fruit Anthocyanin was 120mg//100g.
- Total flavonoid content of Basella alba fruit anthocyanin was also analysed. TFC was found to be 100mg/100g of Basella alba fruit Anthocyanin.
- The presence of antioxidants was first confirmed by checking the total antioxidant activity with the Ascorbic acid standard.
- The % DPPH scavenging activity of rose apple was carried out and it showed high antioxidant activity (69.1%)
- When tested for antimicrobial activity against different organisms, it showed a zone of inhibition of 17mm for Salmonella, 16mm for Staphylococcus aureus and 23mm for E. coli. This proved that the sourced Anthocyanin from Basella alba fruits has very good antibacterial properties.

- Since Basella alba fruit Anthocyanin is high in nutrients, antioxidant activity, and antibacterial properties along with being a natural food colourant, this pigment was incorporated in food products and sensory evaluation was carried out for the pigment incorporated beverage and beverage with its characteristic colour.
- The Basella alba fruit Anthocyanin incorporated Coloured Virgin Mojito scored high scores for appearance, colour, odour, taste, and overall acceptance than the normal Virgin Mojito. The mean score percentage for Coloured Virgin Mojito was 96.8%.

In conclusion, the study emphasizes the development of value-added products from Anthocyanin from different underutilized plant sources due to its pigment colour stability, antioxidant property and antibacterial property. The study revealed good acceptance for the newly developed food products by using Anthocyanin as a natural food colourant.

BIBLIOGRAPHY

BIBLIOGRAPHY

1. (2023, March). Retrieved from Market Data Forecast: https://www.marketdataforecast.com/market-reports/anthocyanins-market
2. Adrian R Whyte, G. S. (2016). Cognitive effects following acute wild blueberry supplementation in 7- to 10-year-old children. *European Journal of Nutrition*, 2151-62.
3. Jiaqi Tan, Y. H. (2022). Extraction and purification of anthocyanins: A review. *Journal of Agriculture and Food Research*.
4. Julia Martín Bueno, P. S.-P.-E. (2012). Analysis and Antioxidant Capacity of Anthocyanin Pigments. Part II: Chemical Structure, Color, and Intake of Anthocyanins. *Critical Reviews in Analytical Chemistry*, 126-151.
5. Katherine Kent, K. C. (2015). Consumption of anthocyanin-rich cherry juice for 12 weeks improves memory and cognition in older adults with mild-to-moderate dementia. *European Journal of Nutrition*, 333-341.
6. Lamport Daniel J 2, L. C. (2016). Concord grape juice, cognitive function, and driving performance: a 12-wk, placebo-controlled, randomized crossover trial in mothers of preteen children. *The American Journal of Clinical Nutrition*, 775-783.
7. Longo, L., Platini, F., Scardino, A., Alabiso, O., Vasapollo, G., & Tessitore, L. (2008). Autophagy inhibition enhances anthocyanin-induced apoptosis in hepatocellular carcinoma. *AACR JOURNALS*, 2476-2485.
8. Luis E. Rodriguez-Saona, R. E. (2001). Extraction, Isolation, and Purification of Anthocyanins. *Current Protocols in Food Analytical Chemistry*.
9. María de Lourdes Vargas y Vargas, J. A. (2013). Extraction and Stability of Anthocyanins Present in the Skin of the Dragon Fruit (Hylocereus undatus). *Food and Nutrition Sciences*, 1221-1228.
10. Massimo Iorizzo 1 2, J. C. (2020). Carrot Anthocyanins Genetics and Genomics: Status and Perspectives to Improve Its Application for the Food Colorant Industry. *PubMed*.

11. Mohammad Imtiyaj Khan, P. S. (2013). Betalains rich Rivina humilis L. berry extract as natural colorant in product (fruit spread and RTS beverage) development. *Journal of Food Science and Technology*.

12. Nazila Ghareaghajlou, S. H.-N. (2021). Red cabbage anthocyanins: Stability, extraction, biological activities and applications in food systems. *ELSEVIER*.

13. Saqib Farooq, M. A. (2020). Recent trends in extraction techniques of anthocyanins from plant materials. *Journal of Food Measurement and Characterization*.

14. Shiyu Li, B. W. (2019). The Anti-inflammatory Effects of Dietary Anthocyanins against Ulcerative Colitis. *PubMed*.

15. Taylor C Wallace, J. B. (2015). Dietary bioactives: establishing a scientific framework for recommended intakes. *PubMed NIH*.

16. Tian Jiang, Y. M. (2018). Degradation of anthocyanins and polymeric color formation during heat treatment of purple sweet potato extract at different pH. *ELSEVIER*.

17. Ute Nöthlings, M. B. (2008). Intake of vegetables, legumes, and fruit, and risk for allcause, cardiovascular, and cancer mortality in a European diabetic population. *The Journal of Nutrition*.

18. WHO. (2004). *Global Strategy on Diet, Physical Activity and Health.* Geneva, Switzerland: WORLD HEALTH ORGANIZATION.

19. Yoon-Mi Lee, Y. Y.-M.-J. (2017). Dietary Anthocyanins against Obesity and Inflammation. *PubMed*.

20. (2006). In K. R. Øyvind M. Andersen, *FLAVANOIDS - CHEMISTRY, BIOCHEMISTRY AND APPLICATION.* Taylor & Francis Group, LLC.

21. Adedotun, I. (2017). Quality Assessment of Oil Extracted from Two Species of Malabar Spinach (Basella alba). *American Scientific Research Journal for Engineering, Technology, and Sciences*.

22. Agnieszka Kumorkiewicz, E. S. (2018). Conjugation of Oxidized Betanidin and Gomphrenin Pigments from Basella alba L. Fruits with Glutathione. *Journal of Agricultural Food Chemistry*.

23. Agnieszka Kumorkiewicz-Jamro, T. Ś.-K. (2021). Multi-colored shades of betalains: recent advances in betacyanin chemistry. *Natural Product Report*.

24. Ahmad Adekilekun Tijani, T. A. (2012). Some of the effects of aqueous leaf extract of Malabar nightshade on the kidney and liver of albino wistar rats. *ResearchGate*.

25. Ahmed A. H. Abdellatif, S. H. (2021). Anthocyanins rich pomegranate cream as a topical formulation with anti-aging activity. *Journal of Dermatological Treatment*.

26. Ajay Chaurasiya, R. K. (2021). An updated review on Malabar spinach (Basella alba and Basella rubra) and their importance . *Journal of Pharmacognosy and Phytochemistry*.

27. Alappat, B. A. (2020). Anthocyanin Pigments: Beyond Aesthetics. *Molecules*.

28. Almedia, M. R. (1996). *Flora of Maharashtra.* Maharashtra: Thomas Paul Almeida for Blatter Herbarium, St. Xavier's College.

29. Ashya Shaik, K. N. (2018). A REVIEW OF ANTHOCYANINS: A PROMISING ROLE ON PHYTOCHEMISTRY AND PHARMACOLOGY. *INTERNATIONAL RESEARCH JOURNAL OF PHARMACY* .

30. Begum, M. &. (2010). First record of Cercospora basellae-albae from the Philippines. *ResearchGate*.

31. Belwal T, N. S. (2017). Dietary Anthocyanins and Insulin Resistance: When Food Becomes a Medicine. *Nutrients*.

32. Bishayee A, H.-R. E. (2010). Anthocyanin -rich black currant extractsuppressesthe growth of human hepatocellular carcinoma cells. *Natural Product Communications*.

33. Bo-Wen Lin, C.-C. G.-F.-Y. (2016). Y. Effects of anthocyanins on the prevention and treatment of cancer. *British Journal of Pharmacology*.

34. CC, N. (2007). Cranberry and its phytochemicals: a review of in vitro anticancer studies. *Journal of Nutrition*.

35. Chang Hui, Y. B. (2010). Anticancer Activities of an Anthocyanin-Rich Extract from Black Rice Against Breast Cancer Cells In Vitro and In Vivo. *Nutrition and Cancer*.

36. Dai J, P. J. (2007). Characterization of blackberry extract and its antiproliferative and anti-inflammatory properties. *Journal of Medicinal Food*.

37. Das, A. M. (2016). Use of Basella alba fruit extract as a potent natural acid-base indicator . *Journal of Chemical and Pharmaceutical Research*.

38. Deka, J. U. (2017). PRELIMINARY PHYTOCHEMICAL SCREENING AND IN VITRO ANTIMICROBIAL ACTIVITY OF ETHANOLIC EXTRACT OF STEM OF THE HERB BASELLA ALBA L. VAR RUBRA (L.) STEWART (FAMILY-BASELLACEAE). *International Journal of Current Pharmaceutical Research,*.

39. Dennis Seyi Arokoyo, I. P. (2018). Antioxidant Activities of Basella alba Aqueous Leave Extract In Blood, Pancreas, and Gonadal Tissues of Diabetic Male Wistar Rats. *Pharmacognosy Research*.

40. Dzierżanowski, A. K. (2021). Targeting Inflammation by Anthocyanins as the Novel Therapeutic Potential for Chronic Diseases: An Update. *Molecules*.

41. Elisa Pojer, F. M. (2013). The Case for Anthocyanin Consumption to Promote Human Health: A Review. *Comprehensive review in Food Scoence and Food Safety*.

42. FAO/WHO. (1982). *Toxicological evaluation of certain food additives.* Geneva: WHO.

43. Gaikwad, S. A. (2014). A review of the taxonomy, ethnobotany, phytochemistry and. *Journal of Applied Pharmaceutical Science*.

44. Gheena S, E. D. (2019). Syringic acid triggers reactive oxygen species–mediated cytotoxicity in HepG2 cells. *Human & Experimental Toxicology.*

45. Gould, K., & Lister, C. (200). In Flavonoids Chemistry, Biochemistry and Applications;. In *Flavonoid functions in plants.* Boca Raton: CRC Press.

46. Grotewold E, D. K. (2008). Trafficking and sequestration of anthocyanins. *Natural Product Communications*.

47. Hong, S. H.-H.-H.-K.-I. (2013). Antidiabetic and Beta Cell-Protection Activities of Purple Corn Anthocyanins. *Korea Science* .

48. Jian He, M. G. (2010). Anthocyanins: Natural Colorants with Health-Promoting Properties. *ResearchGate*.

49. Julia Martín, E. M. (2017). Antioxidant Capacity of Anthocyanin Pigments. *INTEC*.

50. Kähkönen MP, H. M. (2003). Antioxidant activity of anthocyanins and their aglycons. *Journal of Agricultural and Food and Chemistry*.

51. Kevin Gould, K. M. (2009). In *Anthocyanins: Biosynthesis, Functions, and Applications.* Springer.

52. Khan MI, H. P. (2015). Betalains rich Rivina humilis L. berry extract as natural colorant in product (fruit spread and RTS beverage) development. *J Food Sci Technol*.

53. Khan, H. A. (2022). Anthocyanins: Traditional Uses, Structural and Functional Variations, Approaches to Increase Yields and Products' Quality, Hepatoprotection, Liver Longevity, and Commercial Products. *International Journal of Molecular Sciences*.

54. Li Yang, K.-S. W.-X. (2018). Response of Plant Secondary Metabolites to Environmental Factors. *Molecules*.

55. Matsui T, E. S. (2002). Anti-hyperglycemic effect of diacylated anthocyanin derived from Ipomoea batatas cultivar Ayamurasaki can be achieved through the alphaglucosidase inhibitory action. *J Agric Food Chem*.

56. Meiers, S., Kemény, M., Weyand, U., Gastpar, R., von Angerer, E., & Marko, D. (2001). The anthocyanidins cyanidin and delphinidin are potent inhibitors of the epidermal growth-factor receptor. *J. Agric. Food Chem*.

57. Mitra A, D. S. (2016). Use of Basella alba fruit extract as a potent natural acid-base indicator. *Journal of Chemical and Pharmaceutical Research*.

58. Muth, E., Laurent, J., & Jasper, P. (2000). The effect of bilberry nutritional supplementation on night visual acuity and contrast sensitivity. *Altern. Med. Rev*.

59. Newsome AG, C. C. (2014). Nature's palette: the search for natural blue colourants. *J. Agric. Food Chem*.

60. Nollet, L. M. (2000). *Food Analysis by HPLC.* New York: Marcel Dekker Inc.

61. Paola Bontempo, L. D. (2015). Anticancer activities of anthocyanin extract from genotyped Solanum tuberosum L. Vitelotte. *Journal of Functional Foods*.

62. Raymond Brouillard, S. C.-S. (2009). The Visible Flavonoids or Anthocyanins: From Research to Applications. In *Recent Advances in Polyphenol Research.*

63. Regina Cortez, D. A.-V. (2016). Natural Pigments: Stabilization Methods of Anthocyanins for Food Applications. *Comprehensice review in Food Science and Food Safety*.

64. ROSHAN ADHIKARI, N. K. (2012). A REVIEW ON MEDICINAL IMPORTANCE OF BASELLA ALBA L. *International Journal of Pharmaceutical Science and Drugs Research*.

65. S. Oanceaa a*, M. S. (2012). Effects of extraction conditions on bioactive anthocyanin content of Vaccinium corymbosum in the perspective of food applications. *Science Direct*.

66. S. Oanceaa, M. S. (2012). Effects of extraction conditions on bioactive anthocyanin content of Vaccinium corymbosum in the perspective of food applications . *ELSEVIER*.

67. S. Silva, E. M. (2017). Anthocyanin extraction from plant tissues: A review. *Critical Reviews in Food Science and Nutrition*.

68. S. Sravan Kumar, P. M. (2015). Characterization of major betalain pigments gomphrenin, betanin and isobetanin from Basella rubra L. fruit and evaluation of efficacy as a natural colourant in product (ice cream) development. *J Food Sci Technol*.

69. S. Sravan Kumar, P. M. (2015). Nutrition facts and functional attributes of foliage of Basella spp. *ELSEVIER*.

70. S. Sravan Kumar, P. M. (2015). Nutrition facts and functional attributes of foliage of Basella spp. *ELSEVIER*.

71. Sahil Kakkar, S. B. (2014). A Review on Protocatechuic Acid and Its Pharmacological Potential. *International Scholary Research Notes*.

72. Sarikaphuti A, N. T. (2013). Preventive effects of Morus alba L. anthocyanins on diabetes in Zucker diabetic fatty rats. *Exp Ther Med.*

73. Saroj V, R. P. (2012). Pharmacognostical study of Basella alba stem. *Int J Pharm Biol Sci*.

74. Strugała P, D. O.-K. (2019). Antidiabetic and Antioxidative Potential of the Blue Congo Variety of Purple Potato Extract in Streptozotocin-Induced Diabetic Rats. *Molecules*.

75. Suantawee T, E. S. (2017). Cyanidin Stimulates Insulin Secretion and Pancreatic β-Cell Gene Expression through Activation of l-type Voltage-Dependent Ca2+ Channels. *Nutrients*.

76. Subhash Kanti Roy, G. G. (2010). Is stem twining form of Basella alba L. a naturally occurring variant? *JSTOR*.

77. Tanaka Y, S. N. (2008). Biosynthesis of plant pigments: anthocyanins, betalains and carotenoids. *Plant Journal*.

78. Teruo Miyazawa, K. N. (n.d.). Direct Intestinal Absorption of Red Fruit Anthocyanins, Cyanidin-3-glucoside and Cyanidin-3,5-diglucoside, into Rats and Humans. *J. Agric. Food Chem*.

79. Tony K McGhie, M. C. (2007). The bioavailability and absorption of anthocyanins: towards a better understanding. *Molecular Nutrition Food Research*.

80. Valentina Melini, G. P. (2019). Bioactive compounds in rice on Italian market: pigmented varieties as a source of carotenoids, total phenolic compounds and anthocyanins, before and after cooking. *Food Chemistry, ELSEVIER*.

81. XIANLI WU, G. R. (2006). Concentrations of Anthocyanins in Common Foods in the United States and Estimation of Normal Consumption. *Hournal of Agricultural and Food Chemistry*.

82. Yi, W., Fischer, J., & Akoh, C. (2005). Study of Anticancer Activities of Muscadine Grape Phenolics in Vitro. *Journal of Agricultural Fd Chemistry*.

83. Zhenyu Huang, Q. W. (2019). Preliminarily exploring of the association between sugars and anthocyanin accumulation in apricot fruit during ripening. *Scientia Horticulture*.

84. Zhi Yong Ju, L. R. (2007). Effects of solvent and temperature on pressurized liquid extraction of anthocyanins and total phenolics from dried red grape skin. *Journal of Agricultural Food Chemistry*.

85. Gerhäuser, C. (2009). Beer in Health and Disease Prevention. *Sciencedirect*.

APPENDIX

APPENDIX I

SENSORY EVALUATION SHEET

Assessor…… Date……..

(Please score the sample characteristics by placing the relevant score)

Sensory Attributes	**Sample 1**	**Sample 2**
Appearance		
Colour		
Odour		

APPENDIX II

Preparation of reagent for phenolic content determination

- **Folin-Ciocalteau Reagent**

  100g of Folin-Ciocalteau is added to 100ml of distilled water

- **7.5% of Sodium Carbonate**

  Add 75g of sodium carbonate to 100ml of distilled water.

APPENDIX III

Preparation of reagent solution for Flavanoid content determination

- 5% $NaNO_2$

  5g of $NaNO_2$ is made up to 100ml distilled water

- 4% 1M NaOH

  8g of NaOH is made up to 100ml of distilled water

- 10% $AlCl_3$

  10g $AlCl_3$ is made upto 100ml of distilled water

APPENDIX IV

Preparation of reagent solution for Antioxidant activity

- 0.34g Sodium Phosphate
- 0.4943g Ammonium Molybdate
- 3.14ml Sulphuric Acid

All the above Chemicals were made up to 100ml distilled water

ABSTRACT

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The dark blue skin and deep red-violet flesh of Malabar spinach (Basella alba L.) are excellent 29 sources of natural colourant. Basella alba is also known as Malabar spinach, Indian spinach, Ceylon spinach, climber spinach and vine spinach. It is a few meters long succulent, branching, silky, twining herbaceous vine with fruit that is 5–6 mm long, ovoid or spherical, fleshy, stalkless, and purple when matured. It is an underutilized fruit but there are very limited studies done of this species of the Basella genus is done in Kerala. A potential source of natural colourant is the B. alba fruit, which has rich red-violet flesh and a dark blue exterior. Recent research focusing on the extraction of desired bioactive elements from underutilized fruits has attracted a lot of attention for therapeutic applications. To upgrade this natural colourant by replacing the synthetic colourants in the food industry, this study focuses on assessing the stability of the extracted Anthocyanin from Basella alba fruit along with the analysis of its flavonoid and phenol content, antioxidant and antibacterial properties.

The following physical parameters of Basella alba fruit Anthocyanin were determined such as thermal stability and pH stability. Phenol and flavonoid content was also analysed for the extracted Anthocyanin pigment for its use as antioxidant potent natural food colourant. The study also revealed high antioxidant and antibacterial activity. Malabar spinach fruit, being rich in flavonoids and phenols, indigenous value-added products by incorporating Basella alba fruit Anthocyanin developed. A sensory evaluation of the product was also conducted to identify acceptability among consumers.

Keywords: Basella alba, twining herbaceous vine, underutilized, value addition